DUKE UNIVERSITY MEDICAL CENTER

CURRICULUM VITAE

Date Prepared: June 08, 2020

Name (complete with degrees): Sven-Eric Jordt, Ph.D.

Primary academic appointment: Associate Professor, Tenure

Primary academic department (not DUAP): Anesthesiology

Secondary appointment (if any) - (department): Pharmacology & Cancer Biology

Present academic rank and title (if any): Associate Professor, Tenure

Date and rank of first Duke Faculty appointment: Instructor, Temporary

Date of birth: 04/04/1968 Place of birth: (include city/state/country): Lübeck, Germany

Citizen of : Germany

Visa status (if applicable): US Permanent Resident

Education	Institution	Date (Year)	Degree
High School	Johanneum zu Lübeck, Germany	1987	Abitur
College	Free University Berlin, Germany	1994	M.Sc.
Graduate or Professional School	Free University Berlin, Germany	1997	Ph.D.

Scholarly societies (Alpha Omega Alpha, Sigma Xi, Phi Beta Kappa; etc.):

German Academic Scholarship Foundation (Studienstiftung des Deutschen Volkes)

Professional training and academic career (chronologically, beginning with first postgraduate position):

Institution	Position/Title	Dates
Free University Berlin & Center for Molecular Neurobiology Hamburg, Germany Mentor: Thomas Jentsch, M.D., Ph.D.	Graduate Student / Ph.D.	1994-97

Center for Molecular Neurobiology, Hamburg, Germany Mentor: Thomas Jentsch, M.D., Ph.D.	Postdoctoral Fellow	1997-98
Dept. of Cellular & Molecular Pharmacology, University of California, San Francisco (Mentor: David Julius, Ph.D.)	Postdoctoral Fellow	1998-2003
Dept. of Cellular & Molecular Pharmacology, University of California, San Francisco (Mentor: David Julius, Ph.D.)	Postgraduate Researcher	2003-05
Department of Pharmacology, Yale School of Medicine	Assistant Professor, Tenure Track	2005-10
Department of Pharmacology, Yale School of Medicine	Associate Professor, Tenure Track	2010-14
Department of Psychiatry, Yale School of Medicine	Associate Professor, Adjunct	2014-now
Department of Anesthesiology, Duke University School of Medicine (*Primary Appointment*)	Associate Professor, with Tenure	2014-now
Department of Pharmacology & Cancer Biology, Duke University School of Medicine (*Secondary*)	Associate Professor	2017-now

Publications:

1. Refereed journals:

1. **Jordt SE**, Jentsch TJ. Molecular dissection of gating in the ClC-2 chloride channel. ***EMBO J***. 1997;16(7):1582-92. doi: 10.1093/emboj/16.7.1582. PubMed PMID: 9130703; PMCID: PMC1169762.
2. Clark S, **Jordt SE**, Jentsch TJ, Mathie A. Characterization of the hyperpolarization-activated chloride current in dissociated rat sympathetic neurons. ***J Physiol***. 1998;506 (Pt 3):665-78. PubMed PMID: 9503329; PMCID: PMC2230754.
3. Pusch M, **Jordt SE**, Stein V, Jentsch TJ. Chloride dependence of hyperpolarization-activated chloride channel gates. ***J Physiol***. 1999;515 (Pt 2):341-53. PubMed PMID: 10050002; PMCID: PMC2269146.
4. **Jordt SE**, Tominaga M, Julius D. Acid potentiation of the capsaicin receptor determined by a key extracellular site. ***Proc Natl Acad Sci U S A***. 2000;97(14):8134-9. doi: 10.1073/pnas.100129497. PubMed PMID: 10859346; PMCID: PMC16682.
5. Bosl MR, Stein V, Hubner C, Zdebik AA, **Jordt SE**, Mukhopadhyay AK, Davidoff MS, Holstein AF, Jentsch TJ. Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon ClC-2 Cl(-) channel disruption. ***EMBO J***. 2001;20(6):1289-99. doi: 10.1093/emboj/20.6.1289. PubMed PMID: 11250895; PMCID: PMC145530.
6. Chuang HH, Prescott ED, Kong H, Shields S, **Jordt SE**, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. ***Nature***. 2001;411(6840):957-62. doi: 10.1038/35082088. PubMed PMID: 11418861.
7. **Jordt SE**, Julius D. Molecular basis for species-specific sensitivity to "hot" chili peppers. ***Cell***. 2002;108(3):421-30. PubMed PMID: 11853675.
8. **Jordt SE**, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. ***Nature***. 2004;427(6971):260-5. doi: 10.1038/nature02282. PubMed PMID: 14712238.

9. Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED, Julius D, **Jordt SE**, Zygmunt PM. Pungent products from garlic activate the sensory ion channel TRPA1. ***Proc Natl Acad Sci U S A***. 2005;102(34):12248-52. doi: 10.1073/pnas.0505356102. PubMed PMID: 16103371; PMCID: PMC1189336.
10. Bautista DM, **Jordt SE***, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. ***Cell***. 2006;124(6):1269-82. doi: 10.1016/j.cell.2006.02.023. PubMed PMID: 16564016. * (equally contributing first author) **(WoS highly cited paper)**
11. Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, **Jordt SE***, Julius D. The menthol receptor TRPM8 is the principal detector of environmental cold. ***Nature***. 2007;448(7150):204-8. doi: 10.1038/nature05910. PubMed PMID: 17538622. * (co-corresponding author)
12. Boehmerle W, Zhang K, Sivula M, Heidrich FM, Lee Y, **Jordt SE**, Ehrlich BE. Chronic exposure to paclitaxel diminishes phosphoinositide signaling by calpain-mediated neuronal calcium sensor-1 degradation. ***Proc Natl Acad Sci U S A***. 2007;104(26):11103-8. doi: 10.1073/pnas.0701546104. PubMed PMID: 17581879; PMCID: PMC1904151.
13. Streng T, Axelsson HE, Hedlund P, Andersson DA, **Jordt SE**, Bevan S, Andersson KE, Hogestatt ED, Zygmunt PM. Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. ***Eur Urol***. 2008;53(2):391-9. doi: 10.1016/j.eururo.2007.10.024. PubMed PMID: 18031925.
14. Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, **Jordt SE**. TRPA1 is a major oxidant sensor in murine airway sensory neurons. ***J Clin Invest***. 2008;118(5):1899-910. doi: 10.1172/JCI34192. PubMed PMID: 18398506; PMCID: PMC2289796. **(WoS highly cited paper)**
15. Escalera J, von Hehn CA, Bessac BF, Sivula M, **Jordt SE**. TRPA1 mediates the noxious effects of natural sesquiterpene deterrents. ***J Biol Chem***. 2008;283(35):24136-44. doi: 10.1074/jbc.M710280200. PubMed PMID: 18550530; PMCID: PMC2527119.
16. Bessac BF, Sivula M, von Hehn CA, Caceres AI, Escalera J, **Jordt SE**. Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. ***FASEB J***. 2009;23(4):1102-14. doi: 10.1096/fj.08-117812. PubMed PMID: 19036859; PMCID: PMC2660642.
17. Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, Cohn L, Huang X, Moran MM, **Jordt SE**. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. ***Proc Natl Acad Sci U S A***. 2009;106(22):9099-104. doi: 10.1073/pnas.0900591106. PubMed PMID: 19458046; PMCID: PMC2684498. **(WoS highly cited paper)**
18. Lanosa MJ, Willis DN, **Jordt S**, Morris JB. Role of metabolic activation and the TRPA1 receptor in the sensory irritation response to styrene and naphthalene. ***Toxicol Sci***. 2010;115(2):589-95. doi: 10.1093/toxsci/kfq057. PubMed PMID: 20176620; PMCID: PMC2948824.
19. Schulze C, McGowan M, **Jordt SE**, Ehrlich BE. Prolonged oxaliplatin exposure alters intracellular calcium signaling: a new mechanism to explain oxaliplatin-associated peripheral neuropathy. ***Clin Colorectal Cancer***. 2011;10(2):126-33. doi: 10.1016/j.clcc.2011.03.010. PubMed PMID: 21859566; PMCID: PMC3388801.
20. Willis DN, Liu B, Ha MA, **Jordt SE***, Morris JB. Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. ***FASEB J***. 2011;25(12):4434-44. doi: 10.1096/fj.11-188383. PubMed PMID: 21903934; PMCID: PMC3236628. * (corresponding author)
21. Liu B, Escalera J, Balakrishna S, Fan L, Caceres AI, Robinson E, Sui A, McKay MC, McAlexander MA, Herrick CA, **Jordt SE**. TRPA1 controls inflammation and pruritogen responses in allergic contact dermatitis. ***FASEB J***. 2013;27(9):3549-63. doi: 10.1096/fj.13-229948. PubMed PMID: 23722916; PMCID: PMC3752543.

22. Liu B, Fan L, Balakrishna S, Sui A, Morris JB, **Jordt SE**. TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. ***Pain***. 2013;154(10):2169-77. doi: 10.1016/j.pain.2013.06.043. PubMed PMID: 23820004; PMCID: PMC3778045.
23. Gui J, Liu B, Cao G, Lipchik AM, Perez M, Dekan Z, Mobli M, Daly NL, Alewood PF, Parker LL, King GF, Zhou Y, **Jordt SE**, Nitabach MN. A tarantula-venom peptide antagonizes the TRPA1 nociceptor ion channel by binding to the S1-S4 gating domain. ***Curr Biol***. 2014;24(5):473-83. doi: 10.1016/j.cub.2014.01.013. PubMed PMID: 24530065; PMCID: PMC3949122.
24. Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E, Eidam HS, Ye G, Willette RN, Thorneloe KS, Bradshaw HB, Matalon S, **Jordt SE**. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. ***Am J Physiol Lung Cell Mol Physiol***. 2014;307(2):L158-72. doi: 10.1152/ajplung.00065.2014. PubMed PMID: 24838754; PMCID: PMC4152165.
25. Ha MA, Smith GJ, Cichocki JA, Fan L, Liu YS, Caceres AI, **Jordt SE***, Morris JB. Menthol attenuates respiratory irritation and elevates blood cotinine in cigarette smoke exposed mice. ***PLoS One***. 2015;10(2):e0117128. doi: 10.1371/journal.pone.0117128. PubMed PMID: 25679525; PMCID: PMC4334501. * (corresponding author)
26. Smith GJ, Cichocki JA, Doughty BJ, Manautou JE, **Jordt SE**, Morris JB. Effects of Acetaminophen on Oxidant and Irritant Respiratory Tract Responses to Environmental Tobacco Smoke in Female Mice. ***Environ Health Perspect***. 2016;124(5):642-50. doi: 10.1289/ehp.1509851. PubMed PMID: 26452297; PMCID: PMC4858387.
27. Kaelberer MM, **Jordt SE**. A Method to Target and Isolate Airway-innervating Sensory Neurons in Mice. ***J Vis Exp***. 2016(110). doi: 10.3791/53917. PubMed PMID: 27168016; PMCID: PMC4911887.
28. Miao S, Beach ES, Sommer TJ, Zimmerman JB, **Jordt SE**. High-Intensity Sweeteners in Alternative Tobacco Products. ***Nicotine Tob Res***. 2016;18(11):2169-73. doi: 10.1093/ntr/ntw141. PubMed PMID: 27217475; PMCID: PMC5055742.
29. Fan L, Balakrishna S, Jabba SV, Bonner PE, Taylor SR, Picciotto MR, **Jordt SE**. Menthol decreases oral nicotine aversion in C57BL/6 mice through a TRPM8-dependent mechanism. ***Tob Control***. 2016;25(Suppl 2):ii50-ii4. doi: 10.1136/tobaccocontrol-2016-053209. PubMed PMID: 27698211; PMCID:PMC5496986
30. Liu B, Tai Y, Caceres AI, Achanta S, Balakrishna S, Shao X, Fang J, **Jordt SE**. Oxidized Phospholipid OxPAPC Activates TRPA1 and Contributes to Chronic Inflammatory Pain in Mice. ***PLoS One***. 2016;11(11):e0165200. doi: 10.1371/journal.pone.0165200. PubMed PMID: 27812120; PMCID: PMC5094666
31. Liu B, Tai Y, Achanta S, Kaelberer MM, Caceres AI, Shao X, Fang J, **Jordt SE**. IL-33/ST2 signaling excites sensory neurons and mediates itch response in a mouse model of poison ivy contact allergy. ***Proc Natl Acad Sci U S A***. 2016;113(47):E7572-E9. doi: 10.1073/pnas.1606608113. PubMed PMID: 27821781; PMCID: PMC5127381.
32. Peter J, Kasper C, Kaufholz M, Buschow R, Isensee J, Hucho T, Herberg FW, Schwede F, Stein C, **Jordt SE**, Brackmann M, Spahn V. Ankyrin-rich membrane spanning protein as a novel modulator of transient receptor potential vanilloid 1-function in nociceptive neurons. ***Eur J Pain***. 2017;21(6):1072-86. Epub 2017/02/10. doi: 10.1002/ejp.1008. PubMed PMID: 28182310; PMCID: PMC5504413.
33. Caceres AI, Liu B, Jabba SV, Achanta S, Morris JB, **Jordt SE**. Transient Receptor Potential Cation Channel Subfamily M Member 8 channels mediate the anti-inflammatory effects of eucalyptol. ***Br J Pharmacol***. 2017;174(9):867-79. Epub 2017/02/28. doi: 10.1111/bph.13760. PubMed PMID: 28240768; PMCID: PMC5387001.

34. Fait BW, Thompson D, Mose T, Jatlow P, **Jordt S**, Picciotto MR, Mineur YS. Menthol disrupts nicotine's psychostimulant properties in an age and sex-dependent manner in C57BL/6J mice. ***Behav Brain Res***. 2017. Epub 2017/07/27. doi: 10.1016/j.bbr.2017.07.027. PubMed PMID: 28743602

35. Tai Y, Wang C, Wang Z, Liang Y, Du J, He D, Fan X, **Jordt SE**, Liu B. Involvement of Transient Receptor Potential Cation Channel Member A1 activation in the irritation and pain response elicited by skin-lightening reagent hydroquinone. ***Sci. Rep.*** 2017;7(1):7532. Epub 2017/08/10. doi: 10.1038/s41598-017-07651-5. PubMed PMID: 28790335; PMCID: PMC5548750

36. Fait BW, Thompson DC, Mose TN, Jatlow P, **Jordt SE**, Picciotto MR, Mineur YS. Menthol disrupts nicotine's psychostimulant properties in an age and sex-dependent manner in C57BL/6J mice. ***Behav Brain Res.*** 2017; 334:72-77. doi: 10.1016/j.bbr.2017.07.027. Epub 2017 Jul 22. PMID: 28743602; PMCID: PMC5580257

37. Achanta S, Chintagari NR, Brackmann M, Balakrishna S, **Jordt SE**. TRPA1 and CGRP antagonists counteract vesicant-induced skin injury and inflammation. ***Toxicol Lett.*** 2018 Mar 10; doi: 10.1016/j.toxlet.2018.03.007; PubMed PMID: 29535050

38. Zhang L, Terrando N, Xu ZZ, Bang W, **Jordt SE**, Maixner W, Serhan CN, Ji RR. Distinct Analgesic Actions of DHA and DHA-Derived Specialized Pro-Resolving Mediators on Post-operative Pain After Bone Fracture in Mice. ***Front. Pharmacol.*** 2018. ; doi: 10.3389/fphar.2018.00412 PubMed PMID: 29765320

39. Erythropel H, Kong G, de Winter TM, Anastas PT, **Jordt SE**, O'Malley SS, Zimmerman, JB. Presence of high-intensity sweeteners in popular cigarillos of varying flavor profiles. ***JAMA*** 2018;320(13):1380-1383. doi: 10.1001/jama.2018.11187

40. Erythropel HC, Jabba SV, deWinter TM, Mendizabal M, Anastas PT, **Jordt SE***, Zimmerman JB. Formation of flavorant-propylene glycol adducts with novel toxicological properties in chemically unstable e-cigarette liquids. ***Nicotine Tob Res*** 2018 Oct 18. doi: 10.1093/ntr/nty192, *corresponding author

41. Zheng X, Tai Y, He D, Liu B, Wang C, Shao X, **Jordt SE**, Liu B. ETA_R and protein kinase A pathway mediate ET-1 sensitization of TRPA1 channel: A molecular mechanism of ET-1-induced mechanical hyperalgesia. ***Mol Pain***. 2019 Jan-Dec;15:1744806919842473. doi: 10.1177/1744806919842473.

41. Liu B, Tai Y, Liu B, Caceres AI, Yin C, **Jordt SE**. Transcriptome profiling reveals Th2 bias and identifies endogenous itch mediators in poison ivy contact dermatitis. ***JCI Insight***. 2019 Jun 11;5. pii: 124497. doi: 10.1172/jci.insight.124497.

42. Erythropel HC, Davis LM, deWinter TM, **Jordt SE**, Anastas PT, O'Malley SS, Krishnan-Sarin S, Zimmerman JB. Presence and Aerosol-Delivery of Flavor Aldehyde–Solvent Reaction Products, Nicotine, and Menthol in JUUL E-Cigarettes. ***Am. J. Prev. Med.*** (2019) Sep;57(3):425-427. doi: 10.1016/j.amepre.2019.04.004

43. Jabba S, **Jordt SE**. Risk Analysis for the Carcinogen Pulegone in Mint and Menthol-Flavored e-Cigarettes and Smokeless Tobacco Products. (2019) ***JAMA Int. Med.*** (2019) doi: 10.1001/jamainternmed.2019.3649

Submitted Manuscripts:

44. Kaelberer MM, Caceres AI, **Jordt SE**. Activation of a nerve injury transcriptional signature in airway-innervating sensory neurons after lipopolysaccharide induced lung inflammation

Manuscripts in preparation:

45. Achanta S, Chintagari N, Balakrishna S, Liu B, Caceres AI, Kaelberer MM and **Jordt SE.** Accelerating inflammation resolution to counteract chemical cutaneous injury

2. Non-refereed publications: (Refer to those which do not routinely use a system of critical review prior to publication; such articles are often solicited by the publisher.)

n/a

3. Chapters in books:

1. Guimaraes MZP, **Jordt SE** (2007) TRPA1: A sensory channel of many talents. In: Liedtke WB, Heller S, editors. TRP ion channel function in sensory transduction and cellular signaling cascades. Boca Raton, FL: CRC/Taylor & Francis. pp. 151-161.
2. **Jordt, SE** (2007) TRPV1, Regulation by Protons. In Gebhardt GF, Schmidt RF, editors. Encyclopedia of Pain., Berlin, Springer, pp. 2575-2578
3. **Jordt, SE** (2007) TRPV1, Regulation by Nerve Growth Factor, ibid. , pp. 2574-2575

4. **Books:** (Indicate authors or editor.)
   n/a
5. **Non-authored publications:** (Faculty member formally acknowledged in the publication for his/her contributions.)
   n/a
6. **Other:**

a. Published scientific reviews (for mass distribution)

1. **Jordt SE**, McKemy DD, Julius D. Lessons from peppers and peppermint: the molecular logic of thermosensation. ***Curr Opin Neurobiol***. 2003;13(4):487-92. Epub 2003/09/11. doi: S0959438803001016 [pii]. PubMed PMID: 12965298.
2. **Jordt SE**, Ehrlich BE. TRP channels in disease. ***Subcell Biochem***. 2007;45:253-71. Epub 2008/01/16. PubMed PMID: 18193640.
3. Bessac BF, **Jordt SE**. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. ***Physiology (Bethesda)***. 2008;23:360-70. Epub 2008/12/17. doi: 10.1152/physiol.00026.2008. PubMed PMID: 19074743; PMCID: PMC2735846.
4. Rothenberg C, Achanta S, Svendsen ER, **Jordt SE**. Tear gas: an epidemiological and mechanistic reassessment. ***Ann N Y Acad Sci***. 2016;1378(1):96-107. doi: 10.1111/nyas.13141. PubMed PMID: 27391380; PMCID: PMC5096012.
5. Moore C, Gupta R, **Jordt SE**, Chen Y, Liedtke WB. Regulation of Pain and Itch by TRP Channels. ***Neurosci Bull***. 2018;34(1):120-42. Epub 2017/12/29. doi: 10.1007/s12264-017-0200-8. PubMed PMID: 29282613
6. Achanta S, **Jordt SE**.Toxic effects of chlorine gas and potential treatments: a literature review. ***Toxicol Mech Methods.*** 2019 Oct 1:1-13. doi: 10.1080/15376516.2019.1669244. [Epub ahead of print] PubMed PMID: 31532270
7. Gotts JE, **Jordt SE**, McConnell R, Tarran R. What are the Respiratory Effects of E-cigarettes? ***BMJ***. 2019 Sep 30;366:l5275. doi: 10.1136/bmj.l5275. Review. PubMed PMID: 31570493

8. Krishnan-Sarin S, Green B, **Jordt SE** and O'Malley S. WHO Review: The Science of Flavor in Tobacco Products https://www.who.int/publications-detail/who-study-group-on-tobacco-product-regulation-report-on-the-scientific-basis-of-tobacco-product-regulation-seventh-report-of-a-who-study-group

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b. Selected abstracts

Abstracts were presented following committee review at the following meetings:

04.2006 Spring Pain Conference, Grand Cayman (session organizer)
05.2006 Outstanding New Environmental Scientists Symposium, National Institute of Environmental Health Sciences, Durham, NC
04.2007 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
04.2007 Health Effects Institute, Annual Meeting, Chicago
11.2007 Society for Neuroscience, Annual Meeting, San Diego, CA
03.2008 Society of Toxicology Annual Meeting, Seattle, WA
04.2008 Spring Pain Conference, Grand Cayman
04.2008 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
05.2008 American Asthma Foundation, Annual Meeting, San Francisco, CA
10.2008 Society for Neuroscience, Annual Meeting, Minisymposium, Washington, DC (Chair)
04.2009 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
05.2009 American Asthma Foundation, Annual Meeting, San Francisco, CA
05.2009 Society of Toxicology, Annual Meeting, Baltimore, MD
11.2009 Society for Neuroscience, Annual Meeting, Chicago, IL
03.2010 Society of Toxicology Annual Meeting, Salt Lake City, UT
05.2010 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.2010 NIH Countermeasures against Chemical Threats Network Symposium, San Francisco, CA
11.14.2010 Society for Neuroscience, San Diego, CA
02.16.2011 Society for Research on Nicotine and Tobacco (SRNT), Annual Meeting, Toronto
05.12.2011 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.23.2011 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
11.12.2011 Society for Neuroscience, Annual Meeting, Washington, DC
03.12.2012 Society of Toxicology Annual Meeting, San Francisco, CA
05.20.2012 American Thoracic Society, Annual Meeting, San Francisco, CA
06.27.2012 NIH Countermeasures against Chemical Threats Network Symposium, San Francisco, CA
03.14.2013 Society for Research on Nicotine and Tobacco (SRNT), Annual Meeting, Boston
05.19.2013 American Thoracic Society, Annual Meeting, Philadelphia, PA
11.08.2013 Society for Neuroscience, Annual Meeting, San Diego, CA
10.28.2014 New York Academy of Sciences, Pharmacologic Resolution of Inflammation as a Novel Therapeutic Approach, New York, NY
11.02.2014 NIH Tobacco Centers of Regulatory Sciences Grantee Meeting, Bethesda, MD
03.22.2015 Society of Toxicology Annual Meeting, Philadelphia, PA
04.22.2015 Association for Chemoreception Scientists, Ft. Myers, FL
(incomplete)

c. Editorials, position, and background papers

1. **Jordt SE**. Trigeminal TRPs and the scent of pain. ***Pain***. 2011;152(1):4-5. doi: 10.1016/j.pain.2010.10.026. PubMed PMID: 21122996; PMCID: PMC3053048.
2. Achanta S, **Jordt SE**. TRPA1: Acrolein meets its target. ***Toxicol Appl Pharmacol***. 2017. doi: 10.1016/j.taap.2017.03.007. PubMed PMID: 28284857.
3. Summerhill EM, Hoyle GW, **Jordt SE**, Jugg BJ, Martin JG, Matalon S, Patterson SE, Prezant DJ, Sciuto AM, Svendsen ER, White CW, Veress LA. An Official American Thoracic Society Workshop Report: Chemical Inhalational Disasters: Biology of Lung Injury, Development of Novel Therapeutics, and Medical Preparedness. ***Ann Am Thorac Soc***. 2017. doi: 10.1513/AnnalsATS.201704-297WS. PubMed PMID: 28418689.
4. Liu B, **Jordt SE**. Cooling the itch via TRPM8. ***J Invest Derm.*** 2018. doi: 10.1016/j.jid.2018.01.020 PubMed PMID: 29793621
5. **Jordt SE**, Jabba S. Sweeteners are added to modify consumer behaviour. ***BMJ*** . 2019 Jan 25;364:l366. doi: 10.1136/bmj.l366.
6. **Jordt SE**, Jabba S. Tobacco industry's investment in sweetness comes full circle. ***BMJ***. 2019 Jun 10;365:l2338. doi: 10.1136/bmj.l2338.
7. Eissenberg T, Bhatnagar A, Chapman S, **Jordt SE**, Shihadeh A, Soule EK. Invalidity of an Oft-Cited Estimate of the Relative Harms of Electronic Cigarettes. ***Am J Public Health***. 2020 Feb;110(2):161-162. doi: 10.2105/AJPH.2019.305424. PubMed PMID: 31913680
8. Jabba SV, **Jordt SE.** Estimating Fluid Consumption Volumes in Electronic Cigarette Use-Reply. ***JAMA Intern Med.*** 2020 Mar 1;180(3):468-469. doi: 10.1001/jamainternmed.2019.6630. PubMed PMID: 32119051

Statements in the popular press:

09.07.2010 Interviewed by the **Wall Street Journal** for article on cloning of mechanosensory ion channels
http://online.wsj.com/article/SB10001424052748703946504575470033864770518.html#articleTabs%3Darticle

09.06.2011 Interviewed by **The Washington Post** for article on TRP ion channels, natural products, pain and smoking
http://articles.washingtonpost.com/2011-09-05/national/35275560_1_chili-peppers-horseradish-chemical-agent

11.23.2011 Radio Interview on **National Public Radio** about tear gas deployment in Arab Spring uprising in Egypt
http://www.theworld.org/2011/11/tear-gas-egypt/

12.27.2011 Television interview for **HDNet World Report** on tear gas deployment in Arab Spring uprising in Egypt http://vimeo.com/34526996 ; http://www.youtube.com/watch?v=vL7RjxvItfc

04.07.2012 Interviewed by **Science News** magazine for article on new cough therapeutics
http://www.sciencenews.org/view/feature/id/339691/title/Throat_Therapy

11.09.2012 Quoted by **Frankfurter Allgemeine Sonntagszeitung** (leading Sunday Newspaper in Germany) in article on pain and flavor sensing mechanisms in Science/Knowledge section.
http://www.faz.net/aktuell/wissen/natur/pikante-pflanzen-gelobt-sei-was-scharf-macht-11949116.html

06.12.2013 Interviewed by **National Geographic News** about tear gas use in Turkish protests
http://news.nationalgeographic.com/news/2013/06/130612-tear-gas-history-science-turkey-protests/

06.19.2013 Interviewed by **National Geographic News** about pepper spray use against protesters in Brazil
http://news.nationalgeographic.com/news/2013/06/130619-brazil-rio-de-janeiro-pepper-spray-photo-health-impacts/

08.19.2014 Cited in **USA Today** on tear gas health effects
http://www.usatoday.com/story/news/nation-now/2014/08/19/tear-gas-ferguson-chemical-weapons-convention/14279031/

08.21.2014 Interviewed by **New York Magazine** on effects of tear gas
http://nymag.com/scienceofus/2014/08/what-are-the-long-term-effects-of-tear-gas.html

02.15.2015 **Science Daily** highlights study on the effects of menthol in cigarettes
https://www.sciencedaily.com/releases/2015/02/150218092042.htm

11.10.2016 **Scientific American** features Jordt lab study on poison ivy itch and dermatitis
https://www.scientificamerican.com/article/poison-ivy-rsquo-s-itch-can-be-calmed-by-a-protein/
This paper was featured by >25 news outlets, including CBS News, US News and World Report.

04.11.2017 Cited by **Time Magazine** on tear gas use in Venezuela
http://time.com/4735679/venezuela-protest-nicolas-maduro/?iid=sr-link2

02.07.2018 Radio interview, Radio in vivo, WCOM-fm
https://radioinvivo.org/2018/02/07/tear-gas-poison-ivy-and-more/

06.22.2018 Video interview, **Le Monde**, France,
https://www.youtube.com/watch?v=Crjvl4voqns&t=1s

10.18.2018 Featured on **CNN**, **The Verge**, **The Daily Mail**, **The Daily Mirror**, **CBC Canada**
https://www.cnn.com/2018/10/18/health/flavored-e-cigarette-e-liquid-study/index.html
https://www.theverge.com/2018/10/18/17996706/vape-juice-flavoring-electronic-cigarettes-chemicals-ingredients-irritating-e-liquid
https://www.dailymail.co.uk/health/article-6290661/Why-e-cigarette-flavors-dangerous-change-vapors-chemistry.html
https://www.mirror.co.uk/science/e-cigarette-flavourings-react-vaping-13436843
https://www.cbc.ca/news/health/vaping-inhaling-chemicals-second-opinion-october-20-1.4871230

Up to date news contributions by Dr. Jordt can be found here:

https://scholars.duke.edu/person/sven.jordt

Consultant appointments: (Include US government, state, private organizations, etc.)

2006-18 Member, Scientific Advisory Board, Hydra Biosciences LLC, Cambridge, MA
2008 Forrest Laboratories, LLC, Jersey City, NJ
2008 Health Effects Institute, Research Planning Workshop, Boston, MA
2009 Ono Pharmaceuticals, Japan

2011 Cubist Pharmaceuticals, Lexington, MA
2011 Boehringer Ingelheim, Biberach, Germany
2011 Abbott Laboratories, Chicago, IL
2018 Sanofi, Cambridge, MA

Professional awards and special recognitions:

1992-94	Undergraduate Fellowship, German National Merit Foundation, Bonn, Germany
1994	Permanent Fellow, German National Merit Foundation, Bonn, Germany
1998-2001	Postdoctoral Fellowship, German Academy of Sciences, Leopoldina
2003	Young Investigator Award Lecture, International Society for Neurochemistry, Innsbruck, Austria
2006	Outstanding New Environmental Scientist Award (ONES), National Institute of Environmental Health Sciences
2007	Presidential Early Career Award for Scientists and Engineers (PECASE), Office of Science and Technology Policy, Executive Office of the President of the United States
2007	Early Excellence Award, Sandler Foundation for Asthma Research (currently American Asthma Foundation), San Francisco, USA
2010	Extension Award - American Asthma Foundation, San Francisco, USA
2019	Leading Edge in Basic Science Award – Society of Toxicology, Baltimore, USA

Organizations and participation: (Offices held, committee assignments, etc.)

2002-present	Member, Society for Neuroscience (SFN)
2002-present	Member, International Association for the Study of Pain (IASP)
2009-present	Member, Society of Toxicology (SOT)
2010-present	Member, American Thoracic Society (ATS)
2011-present	Society for Research on Nicotine and Tobacco (SRNT), Basic Science Section member
2012-present	Member, Association for Chemoreception Sciences (AChemS)
2012-2013	Delegate, Planning Committee, Environmental and Occupational Health (EOPH) Assembly, American Thoracic Society (ATS)
2013-2019	Delegate, Program Committee, Environmental and Occupational Health (EOPH) Assembly, American Thoracic Society (ATS)
2013-present	Executive Committee, Terrorism and Inhalation Disaster Section, American Thoracic Society
2017-19	Chair, Terrorism and Inhalation Disaster Section, American Thoracic Society (ATS)
2018-19	Executive Committee, Environmental and Occupational Health (EOPH) Assembly, American Thoracic Society (ATS)
2019-present	Regular Member, Program Committee, Environmental and Occupational Health (EOPH) Assembly, American Thoracic Society (ATS)
2019-present	Chair, Nominating Committee, Terrorism and Inhalation Disaster Section, American Thoracic Society (ATS)
2019-present	Co-Chair, Basic Science Network, Society for Research on Nicotine and Tobacco (SRNT)

NIH grant reviewing activity

2009 ZRG1-F02B-Y-20L (NRSA Fellowship Applications, NIH Center for Scientific Review)
09.29.-09.30.2010 ZRG1-IFCN-B-03M, Internet assisted review (R01 applications in sensory biology)
02.22-02.23.2011 ZRG1-IFCN-B-51, Internet assisted review (R01 applications in sensory biology)
06.01-06.02.2011 ZRG1-IFCN-B-02M , Internet assisted review (R01 applications in sensory biology)
03.22.2012 Member, MDCN J54 / J50, CounterACT special emphasis panel, Alexandria, MD
07.19.-07.20.2012 ZAT1 SM (25), NCCAM, Internet assisted review (Asthma mechanisms)
10.22.2012 NIH Intramural Center for Tobacco Regulatory Science
01.31.2013 ZDC1 SRB-R (39). Clinical Research Center Grants (P50), FDA-sponsored tobacco regulatory research
03.19.2013 ZRG1 MDCN-J54 / J50 CounterACT special emphasis panel, Washington, DC
06.04.2013 ZRG1 MDCN-B(55) CounterACT special emphasis panel, Washington, DC
07.11.2014 ZRG1 MDCN-B(55) CounterACT special emphasis panel, Washington, DC
07.16.2015 ZRG1 MDCN-B(55) CounterACT Special Emphasis Panel, Baltimore, MD
07.08.2016 ZRG1 MDCN-B(55) CounterACT Special Emphasis Panel, Baltimore, MD
06.21.2018 ZRG1 MDCN-B(55) CounterACT Special Emphasis Panel, Baltimore, MD
12.04-05.2018 ZRG1-CVRS-N(03): R21 and R01 applications on Electronic Nicotine Delivery Systems: Basic Mechanisms of Health Effects
03.22-23 2019 NIH-CSR-RES SEP-ZRG1 CVRS-H: R21 and R01 applications on Electronic Nicotine Delivery Systems: Basic Mechanisms of Health Effects.
03.27.2019 NCCIH Training and Education Review Panel (CT) Review Panel (ZAT1 AJT 10)
05.30.2019 2019/08 ZRG1 BST-T (55) R RFA Panel: Tobacco Regulatory Biomedical Science - Basic
10.11.2019 ZGM1 RCB-5 (SC) S, NIGMS Support of Competitive Research (SCORE) Program

Grant reviewing for other funders:

07.02.2019 Science Foundation Ireland research grant renewal review, Dublin, Ireland

TO BE COMPLETED FOR CHAIR

Teaching responsibilities including continuing education:

List of Basic Science and Clinical Courses Taught

At Yale University School of Medicine:

		Academic Year / hours									
Course	**Role**	**2005-6**	**6-7**	**7-8**	**8-9**	**9-10**	**10-11**	**11-12**	**12-13**	**13-14**	**14-15**
Bioethics in Neuroscience NSCI 580b [G]	Lecturer	8	3	-	-	3	-	-	-	-	-
Pharmacology 504A [G]	Lecturer	-	16	24	14	10	12	12	12	12	-
Pharmacology 504A [G]	Group discussion leader	-	-	-	-	8	8	8	8	8	-

Seminar in Pharmacology 502 [G]	Course Director	-	45	-	-	-	-	-	40	40	-
Neuropharmacology 508b [G]	Lecturer	-	12	4	4	4	4	4	-	-	-
Principles of Neuroscience NSCI 501a [G]	Lecturer/ Group Discussion Leader	-	-	15	-	-	-	-	-	-	-
Pharmacology, Ethics and responsible conduct in research [G]	Group discussion leader	-	-	-	-	20	4	4	-	-	-
Pharmacology 501 [M]	Lecturer/ conference leader	5	8	22	20	20	20	20	20	-	-
Pharmacology 560b [C]	Lecturer	-	-	-	-	-	-	-	12		-
Pharmacology Graduate Student Laboratory Rotations	Preceptor	-	20		40	-	20	20	-	-	-
Pharmacology Graduate Student Qualifying Exams	Committee member	16	-		-	-	-	-	-	-	-
Interdepartmental Neuroscience Program Rotations [G]	Preceptor	-	-	-	20	-	-	-	-	-	-
MSTP Rotations [G][M]	Preceptor	-	-	-	20	-	-	-	-	-	-
Regulatory & Scientific Issues of Tobacco Use CDE582	Lecturer										8

At Duke University:

		Academic Year / hours		
Course	**Role**	**2017-8**	**2018-19**	**2019-20**
Cellular Signaling- CBI/PHARM/BIOCHEM/MOLCAN 761-1,-2,-3 Module I, II, III	Lecturer	16	16	16

Invited Lectures, CME Courses, Workshops outside of the Medical Center

07.2006 13th Annual Environmental Health Sciences Symposium Dates: July 12-13, 2006, Mt. Desert Island Marine Laboratory, ME
01.2007 University of Southern California, Molecular Biology Seminar Series, Los Angeles, CA
01.2007 Department of Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI
04.2007 Health Effects Institute, Annual Meeting, Chicago

04.2007 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
05.2007 University of Connecticut Health Sciences Center, Farmington, CT
09.2007 International Society for Environmental Epidemiology, Mexico City, Mexico
09.2007 University of Alabama in Birmingham, Department of Anesthesiology Seminar Series
09.2007 American Academy of Physical Medicine and Rehabilitation, Boston, MA (CME)
01.2008 Forest Research Institute, Pharmacology, Jersey City, NJ
03.2008 Society of Toxicology Annual Meeting, Seattle, WA (CME)
04.2008 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
04.2008 International Society for Nephrology - Forefronts Symposium on Polycystic Kidney Disease (PKD), Montreal, Canada
04.2008 Spring Pain Conference, Grand Cayman
05.2008 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.2008 German-American Frontiers of Science Symposium, Humboldt Foundation, National Academy of Sciences USA, Potsdam, Germany
06.2008 Max-Delbrück Center, Research Institute for Molecular Pharmacology, Berlin, Germany
10.2008 Society for Neuroscience, Annual Meeting, Minisymposium, Washington, DC (Chair)
01.2009 Humboldt University, Department of Physiology, Berlin, Germany
02.2009 Duke University, Ion Channel Research Unit Seminar Series, Durham, NC
02.2009 National Institute of Environmental Health Sciences, Laboratory of Respiratory Biology (LRB) Lecture Series, Durham, NC
04.2009 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
05.2009 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.2009 Aspen Lung Conference, Aspen, CO
06.2009 University of Cincinnati, Department of Environmental Health Seminar Series, Cincinnati, OH
09.2009 Life Sciences Summit, University of Stony Brook, NY
09.2009 Lovelace Biomedical and Environmental Research Institute, Second Lovelace CounterACT-CRCE Research Symposium , Albuquerque, NM
03.2010 Society of Toxicology Annual Meeting, Symposium on TRP channel function in chemical sensing (Chair), Salt Lake City, UT
04.2010 Annual Meeting and Conference of the Canadian Society of Biochemistry, Molecular and Cellular Biology, Banff, Canada
05.2010 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.2010 NIH Countermeasures against Chemical Threats Network Symposium, San Francisco, CA
06.2010 Sixth International Cough Symposium, London, UK
06.2010 Ion Channel Retreat, Vancouver, BC, Canada
09.01.2010 National Advisory Environmental Health Sciences Council (NAEHSC), National Institute of Environmental Health Sciences, Research Triangle Park, NC
09.24.2010 TRP Ion Channel Conference, Leuven, Belgium
10.12.2010 Environmental Chemical Threats and Lung Injury: Mechanisms and Countermeasures (CME Workshop), Harvard-Cyprus Initiative for Environment and Public Health, Limassol, Cyprus
10.16.2010 102nd International Titisee Symposium, Boehringer Ingelheim Foundation, Neustadt/Titisee, Germany
11.04.2010 European Rhinitis and Asthma Meeting ERAM, Brussels, Belgium
11.29.2011 University of Vermont Medical College, Burlington, Department of Environmental Pathology
05.2011 American Asthma Foundation, Annual Meeting, San Francisco, CA
06.2011 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
11.14.2010 Society for Neuroscience, San Diego, CA
02.16.2011 Society for Research on Nicotine and Tobacco (SRNT), Annual Meeting, Toronto
05.12.2011 American Asthma Foundation, Annual Meeting, San Francisco, CA

06.23.2011 NIH Countermeasures against Chemical Threats Network Symposium, Washington, DC
10.03.2011 UMDNJ, New Jersey Medical School, Pharmacology & Physiology, Newark, NJ
10.05.2011 University of Connecticut, Pharmaceutical Sciences, Storrs, CT
11.12.2011 Society for Neuroscience, Annual Meeting, Washington, DC
01.30.2012 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
03.12.2012 Society of Toxicology Annual Meeting, San Francisco, CA
04.25.2012 Columbia University Medical Center, Skin Disease Research Center, New York, NY
05.20.2012 American Thoracic Society, Annual Meeting, San Francisco, CA (CME)
06.01.2012 Neurocolloquium, Charite School of Medicine, Berlin, Germany
06.27.2012 NIH Countermeasures against Chemical Threats Network Symposium, San Francisco, CA
05.19.2012 American Thoracic Society, Annual Meeting, Philadelphia, PA (CME)
09.08.2012 Society of General Physiologists, 66th Annual Symposium, MBL, Woods Hole, MA
09.12.2012 International Workshop on Transient Receptor Potential Ion Channels, Valencia, Spain
03.14.2013 Society for Research on Nicotine and Tobacco (SRNT), Annual Meeting, Boston
03.26.2013 University of Alabama, Birmingham, Dept. of Anesthesiology
04.12.2013 Brown University, Department of Molecular Pharmacology, Physiology and Biotechnology
08.28.2013 University of Pittsburgh, Dept. of Environmental Health, Center for Pain Research
09.04.2013 University of Vermont, Dept. of Pharmacology, Burlington, VT
09.16.2013 Case Western Reserve University, Dept. of Pharmacology, Cleveland, OH
03.27.2014 Monell Chemical Senses Center, Philadelphia, PA
06.17.2014 NIH Countermeasures against Chemical Threats Network Symposium, Denver, CO
07.01.2014 Outstanding Environmental Scientists Symposium, NIEHS, Raleigh, NC
09.18.2014 Society for Research on Nicotine and Tobacco Europe, Santiago de Compostela, Spain
10.02.2014 University of North Carolina, Cell Biology & Physiology, Chapel Hill, NC
02.20.2015 FDA Center for Tobacco Products (FDA-CTP), Bethesda, MD
02.25.2015 FDA Workshop on Tobacco Regulatory Science at Society for Research on Nicotine and Tobacco Annual meeting, Philadelphia, PA
05.20.2015 American Thoracic Society, NIEHS Session, Environmental Chemicals, Denver, CO
06.02.2015 FDA 3rd Public Workshop on Electronic Cigarettes, College Park, MD
06.15.2015 NIH Countermeasures against Chemical Threats Network Symposium, New York, NY
11.05.2015 Science Day, National Institute of Environmental Health Sciences (NIEHS), Raleigh, NC
03.14.2016 Society of Toxicology, Workshop on TRP Ion Channels, New Orleans, LA
05.16.2016 American Thoracic Society, Symposium of the Terrorism and Inhalation Disaster Section (Organizer), San Francisco, CA
06.15.2016 NIH Countermeasures against Chemical Threats Network Symposium, Davis, CA
09.08.2016 Tobacco Center of Regulatory Science Retreat, University of North Carolina, Chapel Hill, NC
09.29.2016 International Symposium on TRP Ion Channels, Munich, Germany
02.22.2017 Depts. of Immunology, Neuroscience, Genentech, South San Francisco, CA
03.08.2017 Department of Biochemistry, University of Florence, Italy
03.09.2017 FDA Symposium at Society for Research on Nicotine and Tobacco International Meeting, Florence, Italy
03.10.2017 Podium Presentation, Society for Research on Nicotine and Tobacco International Meeting, Florence, Italy
04.05.2017 Medical Chemical Defense Conference, German Armed Forces University, Munich, Germany
04.27.2017 Association for Chemosensation Sciences Annual Meeting, Bonita Springs, FL
06.01.2017 Department of Physiology, University of Tennessee Health Science Center, Memphis, TN

06.12.2017 NIH Countermeasures against Chemical Threats Network Symposium, Boston, MA
06.18.2017 Department of Neurobiology, Zhejiang Chinese Medical University, Hangzhou, China
06.21.2017 Translational Pain Research Symposium, Duke Kunshan University, China
11.11.2017 Nanosymposium: Touch, Itch & Pain, Society for Neuroscience Annual Meeting, Washington, DC
11.12.2017 Minisymposium: Peripheral Neural Modulation of Inflammation, Society for Neuroscience Annual Meeting, Washington, DC
02.23.2018 Symposium: Cooling Agents, Flavors and Nicotine: Additives or Drugs ? Society for Research on Nicotine and Tobacco Annual Meeting, Baltimore, MD
03.22.2018 Virginia Conference on Youth Tobacco Use, Virginia Commonwealth University, Richmond, VA
04.27.2018 Strategic Asthma Basic Research Center (SABRE), University of California, San Francisco, CA
08.18.2018 Department of Molecular Biomedical Sciences, NC State Veterinary Medicine, Raleigh, NC
09.08.2018 Oral Session: Biological Impact of Tobacco Smoke and E-Vapor (Chair, Speaker), Society for Research on Nicotine and Tobacco Europe (SRNT-E) Annual Meeting, Munich
11.15.2018 Organization for the Prohibition of Chemical Weapons (OPCW), Temporary Working Group on Investigative Science and Technology Meeting, The Hague, The Netherlands
12.18.2018 Workshop Sensory Pharmacology, Sanofi, Cambridge, MA
03.05.2019 Gordon Research Conference - Chemical and Biological Terrorism Defense, Ventura, CA
03.13.2019 Leading Edge in Basic Science Award Lecture, Annual Meeting, Society of Toxicology, Baltimore, MD
03.26.2019 Inflammation Resolution Biology Workshop, National Institute of Environmental Health Sciences (NIEHS), RTP, NC
04.24.2019 A Tobacco Regulatory Agenda for Vulnerable and Disparate Groups: Developing a Blueprint for Research, Policy, and Regulatory Standards, University of Arkansas Little Rock, AK
05.19.2019 Chair, Minisymposium Chemical Threats and Injury: Mechanisms and Treatment M Dallas Downtown
05.23.2019 Toxicology Graduate Program Seminar Series, Department of Pharmacology, LMU University Munich, Germany
11.17.2019 International Forum for the Study of Itch (IFSI), Annual Meeting, Sydney, Australia
02.25.2020 Department of Anatomy and Physiology, College of Veterinary Medicine. Kansas State University, Manhattan, KS
03.03.2020 Symposium: Molecular Mechanisms of Chemical Sensing, 5th German PharmTox Summit, German Society for Pharmacology and Toxicology (DGPT). Leipzig, Germany

Major Lectures, Seminars,Workshops at School of Medicine

Yale School of Medicine
04.21.2006 Lung Biology Seminar Series, Pulmonary Section, Department of Internal Medicine
10.25.2007 Developmental Therapeutics Conference, Yale Cancer Center
12.04.2007 Vascular Biology and Therapeutics Seminar, VBT Program (CME)
02.18.2009 Center for Neuroscience and Regeneration Research, VA Hospital, West Haven
02.20.2009 Lung Inflammation Group, Pulmonary Section / Dept. of Immunobiology
03.30.2010 Pulmonary Research Conference, Pulmonary Section, Department of Internal Medicine
09.17.2010 Lung Inflammation Group, Pulmonary Section / Dept. of Immunobiology
11.22.2010 Biological Sciences Training Program (BSTP) Lecture / Dept. of Psychiatry
02.13.2012 Pierce Laboratories Seminar Series

Duke University School of Medicine
07.16.2014 Duke Pain Seminar
10.01.2014 Duke Integrated Toxicology and Environmental Health Program (ITEHP)
01.20.2016 Grand Rounds, Department of Anesthesiology
09.11.2017 Department of Pharmacology, Seminar
10.08.2018 Center for Perioperative Organ Protection Seminar, Department of Anesthesiology
01.08.2019 Pinnell Center for Investigative Dermatology, Department of Dermatology
09.27.2019 Duke Integrated Toxicology and Environmental Health Program (ITEHP)

Areas of research interests (basic and applied) - list:

1 TRP ion channels in environmental chemical sensing

The sensory neurons innervating the respiratory system are similar to the pain-transducing sensory neurons I studied in my postdoctoral research. Peripheral sensory nerve fibers, derived from sensory neurons of the dorsal root, trigeminal, nodose and jugular ganglia, innervate all interior organs, as well as the skin and mucous membranes in the eyes and airways. These neurons are divided into subpopulations that transmit chemical (acids, bases, reactive chemicals, natural products) and physical (thermal, mechanical) interior and environmental stimuli to elicit the sensations of pain, irritation, heat and cold, as well as pressure. The goal of the research in the Jordt laboratory is to identify the receptors and mechanisms that enable sensory neurons to detect chemical and physical stimuli, and to elucidate their roles in respiratory physiology and disease.

We initially focused on the role of sensory TRP ion channels, a family of ion channels activated by heat, cold and chemical signals. For these studies we are applying molecular, electrophysiological, imaging, behavioral and genetic approaches. Recently, we discovered that the sensory ion channel, TRPA1, is the major receptor for reactive environmental toxicants and inflammatory agents in sensory neurons innervating the airways. TRPA1 is activated by a multitude of electrophilic or oxidizing chemical stimuli through reactive protein modification, leading to opening of the channel pore. Using TRPA1-deficient mice we demonstrated that TRPA1 is essential for the initiation of respiratory reflexes in mice that are equivalent to cough and airway constriction in humans. In detailed cellular and animal physiological studies we found that TRPA1 is activated by acrolein, a major irritant constituent of tobacco smoke and photochemical smog, and by highly irritating chlorine gas. In ongoing studies we examine the effects of TRPA1 antagonists on chemical irritation and airway and cutanous injury. These studies revealed that TRPA1 antagonists are highly effective in blocking pain responses to tear gas agents and other highly potent chemical irritants. The discovery of TRPA1 as the major sensory irritant receptor represented a significant breakthrough in respiratory biology and medicine, environmental health sciences and toxicology, and was recognized as such by the Presidential Early Career Award (PECASE).

2 Role of chemosensory TRP ion channel in asthma and atopic disease

Studies over the last 40 years point to an essential role of peripheral sensory neurons in the initiation and maintenance of chronic inflammation in conditions such as asthma, allergic and atopic dermatitis and arthritis. However, it has remained unclear how immunogenic signals are detected by sensory neurons and how sensory neurons contribute to inflammation. The most recent work in the Jordt laboratory revealed that sensory TRP channels are targets of endogenous chemical mediators produced during injury and inflammation by eosinophils, neutrophils and other cells of the immune system. For example, we observed that TRPA1 is activated by reactive oxygen species and oxidative lipid products, thereby initiating inflammatory pain. Thus, TRPA1 may represent a candidate target for immunogenic mediators to initiate pain and neurogenic inflammatory mechanisms. We tested this hypothesis in a mouse model of allergic asthma, observing that TRPA1-deficient mice failed to develop allergic airway inflammation and airway hyperreactivity reminiscent to asthmatic bronchoconstriction in humans. These results were recapitulated using a small molecule TRPA1 antagonist. We established key methods for the

study of pulmonary inflammation and reactivity, including forced oscillation analysis of pulmonary resistance, elastance and compliance, and immunological and pathological methods. The discovery of the involvement of sensory neuronal receptors in asthma was acknowledged by the Early Excellence Award to Dr. Jordt by the American Asthma Foundation (formerly Sandler Foundation for Asthma Research), followed by an extension award by the Foundation.

3 Anti-inflammatory and analgesic therapeutics, novel anti-inflammatory targets and in vivo imaging of inflammation

We continue collaborations with GlaxoSmithKline Pharmaceuticals and Hydra Biosciences, a biotechnology company in Cambridge, MA, to develop TRP ion channel inhibitors as anti-inflammatory treatments for asthma and other allergic and atopic conditions. Current research focuses on the role of sensory neurons in allergic contact dermatitis and atopic dermatitis using hapten-induced mouse models. The goal of these studies is to investigate the role of sensory neurons and cutaneous factors in the atopic march leading to asthma. New studies apply comprehensive molecular methods such as RNAseq of skin cell populations, of sensory neurons labeled by GFP expression and pulmonary tissue to examine the presence and transcriptional regulation of key mediators of cutaneous and pulmonary inflammation and neuronal receptors and peptides in murine and human tissues. We also use infrared fluorescent imaging technique to detect tissue injury and inflammation in the skin of mice. We specifically focus on mouse models of skin blistering diseases in which skin edema formation and inflammation are imaged. Recent studies have focused on the refinement of translational animal models of pruritic dermatitis, specifically a poison ivy model of allergic contact dermatitis. These models allowed us to reveal novel signaling pathways promoting inflammation and itch of translational relevance.

4 Development of countermeasures against acute lung injury

Since 2006 my laboratory has conducted systematic studies to identify novel treatments for acute lung injury induced by chemical exposures or inflammatory agents. This research originated in our discovery of TRP ion channels as key sensors for chemical irritants in the respiratory system. Supported by grants from NIH's Countermeasures against Chemical Threats (CounterACT) program, we established a network of academic collaborators and contractors for testing candidate treatments for lung injury induced by exposures to chlorine or sulfur mustard in mice and large animal models. Together with industry (GlaxoSmithkline Pharmaceuticals, Hydra Biosciences) and academic collaborators (Dr. Sadis Matalon at the University of Alabama in Birmingham), we tested the actions of TRP channel inhibitors in mice exposed to chlorine gas. In these studies we identified several small molecule drug candidates as potent inhibitors of edema formation, vascular leakage and inflammation. These treatments resulted in improved oxygen saturation and other correlates of survival. At Duke we translated these outcomes to a large animal model, the chlorine exposed pig, replicating efficacy and potency of TRP inhibition. This effort has resulted in a successful pre-IND consultation with FDA and a successful application by GSK for drug development support by the Biomedical Advanced Research and Development Authority (BARDA).

5 Studies addressing FDA priorities for research on tobacco products

The Family Smoking Prevention and Tobacco Control Act (FSPTCA) empowered the FDA to control the composition of tobacco products to reduce the hazards of smoking and tobacco smoke exposures. A priority for FDA is research on the effects of menthol in tobacco products. In the United States, almost all adolescent smokers initiate smoking with mentholated cigarettes. The effects of menthol on smoking initiation and addiction remain poorly understood. Menthol activates TRPM8, a cold-sensing ion channel in sensory neurons that mediate the sensation of cool. To address FDA's research priorities the Jordt laboratory leveraged the extensive expertise in the pharmacology and physiology of TRPM8 and identified novel agonists and antagonists for this ion channel. The Jordt laboratory, in collaboration with Dr. John Morris at the University of Connecticut, analyzed the ventilatory patterns in mice to demonstrate that menthol inhibits the neuronally mediated respiratory irritation

response to tobacco smoke irritants in mice. These data suggest that menthol facilitates smoke inhalation resulting in increased nicotine and smoke toxicant exposures. Current experiments focus on the effects of menthol and other flavorant chemicals in wild-type mice and TRP channel-deficient mice. Further investigations, funded through a FDA/NIDA-supported Tobacco Center of Regulatory Science (TCORS) and a R01 (NIEHS), investigate the pharmacological effects of electronic cigarette vapor constituents and their toxicological properties.

External support - gifts, grants, and contracts:

a) **Past:**

Agency: Health Effects Institute
ID#: -
Title: Ion Channels in Airway Sensory Nerve Endings as Mediators of the Irritant Effects of Acrolein
P.I.: Sven-Eric Jordt, Ph.D.
Grant period: 05/01/2006-04/31/2007

Agency: Department of Public Health, State of Connecticut
ID#: 2007-0161 BIOMED
Title: Sensory Irritant Receptors in the Pathogenesis of Smoking-Induced Lung Disease
P.I.: Sven-Eric Jordt, Ph.D.
Grant period: 07/01/2006-12/31/2008

Agency: American Asthma Foundation (formerly Sandler Foundation for Asthma Research)
ID#: -
Title: Sensory Chemoreceptors in Asthma and Airway Hyperresponsiveness
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 08/01/2007 – 07/31/2010

Agency: American Asthma Foundation (formerly Sandler Foundation for Asthma Research)
ID#: 10-JORD-EX1
Title: Sensory Chemoreceptors in Asthma and Airway Hyperresponsiveness (Extension)
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 07/01/2010 – 06/30/2011

Agency: NIH/NINDS
ID#: R21NS058330
Title: Novel Analgesics from Australian Funnel-Web Spider Venom
P.I.: Michael Nitabach, Ph.D., J.D., Department of Physiology, Yale University School of Medicine
Role: Co-PI
Project period: 05/15/2009 – 04/30/2011

Agency: NIH/NIEHS
ID#: U54 NS063739
Title: Mechanisms of Chlorine Hypersensitivity in Asthma (Sub-project)
P.I.: Sadis Matalon, Ph.D., University of Alabama, Birmingham, AB

Role: Sub-project leader

Agency: NIH/NIEHS
ID#: U01 ES015674 S1 (Supplement)
Title: Targeting Injury Pathways to Counteract Pulmonary Agent and Vesicant Toxicity
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 09/01/2012 – 05/31/2013

Agency: NIH/NIEHS
ID#: R01 ES015056
Title: TRPA1 Channels in Sensory Neurons as Targets for Environmental Irritants
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 09/01/2006 – 10/31/2013

Agency: NIH/NIEHS
ID#: R21 ES022875
Title: Accelerating Inflammation Resolution to Counteract Chemical Injury
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 09/19/2012 – 08/31/2015

Agency: NIH/NHLBI
ID#: R01 HL105635
Title: Counterirritation by Menthol: Molecular Targets and Role in Airway Disease
P.I.: Sven-Eric Jordt, Ph.D.
Percent Effort: 20%
Direct costs per year: $ 280,700
Total costs for project period: $ 1,624,326
Project period: 01/01/2011 – 12/31/2015

Agency: NIH/NHLBI
ID#: R01 HL105635 S1 (Supplement)
Title: Counterirritation by Menthol: Molecular Targets and Role in Airway Disease
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 09/14/2012 – 12/31/2015

Agency: NIH/NIDA/FDA
ID#: P50DA036151S1
Title: Irritant Flavor Products in Heated E-Cigarette Liquids and Vapors
P.I.: Suchitra Krishnan-Sarin, Stephanie O'Malley
Role: Supplement Project Director
Project period: 09/01/2016 – 08/31/2016

Agency: NIH/NIEHS
ID#: U01 ES015674
Title: Targeting Injury Pathways to Counteract Pulmonary Agent and Vesicant Toxicity
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 09/29/2006 – 05/31/2018

Agency: NIH/NIAMS
ID#: R21AR070554
Title: Mechanisms of Itch in Poison Ivy-Induced Allergic Contact Dermatitis
P.I.: Sven-Eric Jordt, Ph.D.
Project period: 07.01.2016-06.30.2018

b) **Present:**

Agency: NIH/NIEHS
ID#: R01ES029435
Title: Anesthetic and synthetic cooling flavors in E-cigarettes: Chemistry and respiratory effects modulating nicotine intake
PI: Sven-Eric Jordt, Ph.D.
Project period: 04/01/2018 – 03/31/2021

Agency: NIH / NIEHS
ID#: U01ES030672
Title: Advanced TRPA1 Inhibitor for the Treatment of Chlorine Inhalation Injury
Role: Multi-PI
Percent Effort: 25%
Project period: 07/01/2019-06/30/2022

Agency: NIH/NIEHS
ID#: U54ES027698
Title: Development of Antidotes for Toxic Gases
P.I.: Carl White, M.D.
Role: Significant Contributor (Subcontract)
09/30/2016-08/31/2020

Agency: NIH/NIDA/FDA
ID#: U54DA036151
Title: Yale Center for the Study of Tobacco Product Use and Addiction: Flavors, Nicotine and Other Constituents (YCSTP)
P.I.: Suchitra Krishnan-Sarin, Ph.D., Stephanie O'Malley, Ph.D.
Role: Project Director, Project 1
Project period:09/01/2018 – 08/31/2023

Agency: NIH / NIEHS
ID#: R21ES030331
Title: Specialized pro-resolving mediators as potential medical countermeasures in a pig model of chlorine gas-induced acute lung injury
Role: Multi-PI
Project period: 07/01/2020-06/30/2022

Agency: NCCIH
ID#: P01AT009968
Title: Resolution of Neuroinflammation and Persistent Pain by ComplementaryApproaches

P.I.: William Maixner, D.D.S., Ph.D.
Role: Multi-PI, Project 3; PI Molecular Core
Percent Effort: 15% ; 10%
Direct costs per year: $185,000 ; $23,282
Total costs for project period: TBD
Project Period: TBD
Status: In revision

Participation in academic and administrative activities of the University and Medical Center

2014- Department of Anesthesiology, Research Council
2017- Reviewer for Duke Bridge Funding Committee
2018- Member, APT Committee, Department of Anesthesiology
2019- Associate Research Quality Officer, Department of Anesthesiology

Jordt Lab - Past Predoctoral Trainees

Trainee Name	Training Period	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Prior Academic Degree Institution	Title of Research Project	Research Support	Current Position
Michael Sivula	09.06-12.08	B.S.	2002	University of Virginia	TRPA1 is a Peripheral Neuronal Sensor for Oxidants and Lipid Peroxidation Products	Pharmacology Program, Yale School of Medicine	Senior Staff Scientist, Therapeutic Proteins, Regeneron Pharmaceuticals, Terrytown, NY
Christian von Hehn	12.06-12.09	M.D.	2003	University of Kiel, Germany	TRPA1 as a Sensory Neuronal Target of Chemical Irritants and Counterirritants	Interdepartmental Neuroscience Program, Yale University	Medical Director, Clinical Development, Biogen, Weston, MA
Jasmine Escalera	06.06-05.10	B.S.	2004	Pace University	Terpene ligands of sensory TRP channels - structure and function	F31 ES015932 pre-doctoral fellowship (NIH)	Program & Operations Manager, New York University, New York, NY
Maxwell Elia	03.08-05.11	B.A. M.S.	2007	University of Pennsylvania	Inflammation control by sensory neurons	Yale Medical Scientist Training Program (MSTP) , MD/PhD	Ophthalmologist, Retina and Eye Disease Fellow, Yale New Haven Hospital
Melanie Kaelberer	05.10-05.16	B.S.	2006	UCSD	Regulation of Sensory Receptors and Mediators in Asthma	Physiology Program, Yale School of Medicine	Postdoctoral Associate, Dept. of Gastroenterology, Duke University School of Medicine

Jordt Lab - Past and Current Postdoctoral Trainees

Trainee Name	Training Period	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Prior Academic Degree Institution(s)	Title of Research Project	Research Support	Current Position
Bret Bessac	02.06.-06.10	Ph.D.	2005	University of Hawaii	Sensory neurons and respiratory reflexes	Dept Pharmacology, T32 Neuropharma-cology Training Program	Assistant Professor, Department of Pharmaceutical Sciences Texas Tech University Health Science Center School of Pharmacy Dallas, TX
Marian Brackmann	04.07-10.09	Ph.D.	2004	Free University, Berlin, Germany	Accessory subunits of sensory TRP channels	Postdoctoral Fellowship, German Research Foundation, 09.08-08.09	Project Coordinator, University of Applied Sciences, Bielefeld, Germany
Yi-Shiuan Liu	03.07-09.09	Ph.D.	2004	University of Virginia, Charlottesville	Transgenic neuronal labeling with fluorescent calcium sensors		Assistant Professor, Department of Physiology and Pharmacology, Chang Gung University, Taoyuan, Taiwan
Ana Caceres	04.07-03.12	Ph.D.	2007	University of Valladolid, Spain	TRPA1 in asthma and airway hyperreactivity	MEC-Fulbright Postdoctoral Fellowship, Ministry of Education and Science, Spain, 09.07-08.09	
Ruchira Singh	03.09.-01.12	Ph.D.	2009	Kansas State Univ.	TRP channels in diabetic peripheral neuropathy		Assistant Professor of Ophthalmology & Biomedical Genetics, University of Rochester, NY
Shrilatha Balakrishna	04.09-03.14	Ph.D.	2007	University of Mysore, India	Neuronal mechanisms of chemical hypersensitivity in inflammation		Research Liaison, Office of Animal Research Support (OARS), Yale University
Boyi Liu	11.09-03.16	Ph.D.	2009	Hebei Medical University, China	Mechanisms of pain, itch and analgesia	DREAM Innovation Grant, Dept. of Anesthesiology	Professor, Zhejiang Chinese Medical University, Hangzhou, China

Trainee Name	Training Period	Prior Academic Degree(s)	Prior Academic Degree Year(s)	Prior Academic Degree Institution(s)	Title of Research Project	Research Support	Current Position
Lu Fan	08.10-03.14	M.D. Ph.D.	2008	University of Illinois, Chicago	Oxidative stress and neuropathic pain		Resident Physician, Nassau University Medical Center, NY
Narendranath Chintagari	12.10-07.12	Ph.D.	2007	Oklahoma State Univ., Stillwater, OK	Mechanisms of vesicant injury		Staff Fellow, Food and Drug Administration (FDA), Bethesda, MD
Satya Achanta	04.12-03.14	D.V. M. Ph.D.	2012	Oklahoma State Univ.	Countermeasures against chlorine inhalation injury		Assistant Professor of Anesthesiology, Duke University School of Medicine
Sairam Jabba	03.15-06.18	Ph.D.	2012	Creighton Univ., OK	Tobacco regulatory science		Senior Research Associate, Duke Univ.

The FASEB Journal • Research Communication

Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases

Bret F. Bessac, Michael Sivula,[1] Christian A. von Hehn,[1] Ana I. Caceres, Jasmine Escalera, and Sven-Eric Jordt[2]

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut, USA

ABSTRACT **The release of methyl isocyanate in Bhopal, India, caused the worst industrial accident in history. Exposures to industrial isocyanates induce lacrimation, pain, airway irritation, and edema. Similar responses are elicited by chemicals used as tear gases. Despite frequent exposures, the biological targets of isocyanates and tear gases *in vivo* have not been identified, precluding the development of effective countermeasures. We use Ca^{2+} imaging and electrophysiology to show that the noxious effects of isocyanates and those of all major tear gas agents are caused by activation of Ca^{2+} influx and membrane currents in mustard oil-sensitive sensory neurons. These responses are mediated by transient receptor potential ankyrin 1 (TRPA1), an ion channel serving as a detector for reactive chemicals. In mice, genetic ablation or pharmacological inhibition of TRPA1 dramatically reduces isocyanate- and tear gas-induced nocifensive behavior after both ocular and cutaneous exposures. We conclude that isocyanates and tear gas agents target the same neuronal receptor, TRPA1. Treatment with TRPA1 antagonists may prevent and alleviate chemical irritation of the eyes, skin, and airways and reduce the adverse health effects of exposures to a wide range of toxic noxious chemicals.—Bessac, B. F., Sivula, M., von Hehn, C. A., Caceres, A. I., Escalera, J., Jordt, S.-E. Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. *FASEB J.* 23, 1102–1114 (2009)**

Key Words: sensory neurons · pain · nociception · analgesia · TRP channels

Isocyanates are reactive organic chemicals widely used in the industrial production of polyurethane polymers, pesticides, fungicides, and other materials. Methyl isocyanate (MIC), a precursor in pesticide production, was the major causative agent of the environmental disaster in Bhopal, India, responsible for more than 3000 immediate deaths and several thousand additional casualties in the years after the accident (1). In the United States, MIC exposures have occurred after spills of the pesticide metam sodium (sodium *N*-methyl-dithiocarbamate) in railroad and agricultural accidents (2, 3). In these accidents, metam sodium reacted with soil components and water to produce MIC and other reactive agents (3–5). MIC exposure caused immediate unbearable irritation of eyes, nose, and throat (6). The airways are especially sensitive to MIC and other isocyanates. Dependent on exposure levels and duration, MIC-exposed individuals present with airway hyperresponsiveness, inflammation, reactive airway dysfunction syndrome (RADS), and airway edema and injuries (1). Bifunctional isocyanates such as 2,4-toluene-diisocyanate, diphenylmethane-4,4′-diisocyanate (MDI), and hexamethylene-diisocyanate (HDI), used in the production of polyurethane products, are equally strong irritants and cause asthma-related symptoms on repeated exposures (7).

The severe irritation after exposures to isocyanates is surprisingly similar to the incapacitating effects of tear gas agents (8, 9). The development of tear gas agents dates back to World War I, when almost all factions used airway irritants and chemical lacrimatory agents (tear gases), such as acrolein (Papite), chloropicrin (PS), bromoacetone, benzyl bromide, and others (9–11). CN tear gas, a riot control agent, was developed in the 1920s and was widely used by law enforcement until the 1960s (12). The active lacrimatory agent in CN is 2-chloroacetophenone. Because of its toxicity, CN was supplanted by CS tear gas, containing 2-chlorobenzylidene malononitrile as its active ingredient. CS is currently the most widely used riot control agent worldwide. CR is another modern riot control agent, containing dibenzo[*b,f*][1,4]oxazepine as its lacrimatory principle (**Fig. 1*B***) (12).

Despite the infamy of isocyanate exposures in occupational and environmental medicine and the widespread and frequent use of tear gas agents for more than 90 yr, with possibly millions of exposures, little is known about the molecular and cellular actions of these agents. Current medical treatment of exposures includes the removal of the toxicants by dilution, washing, and chemical neutralization, treatment of

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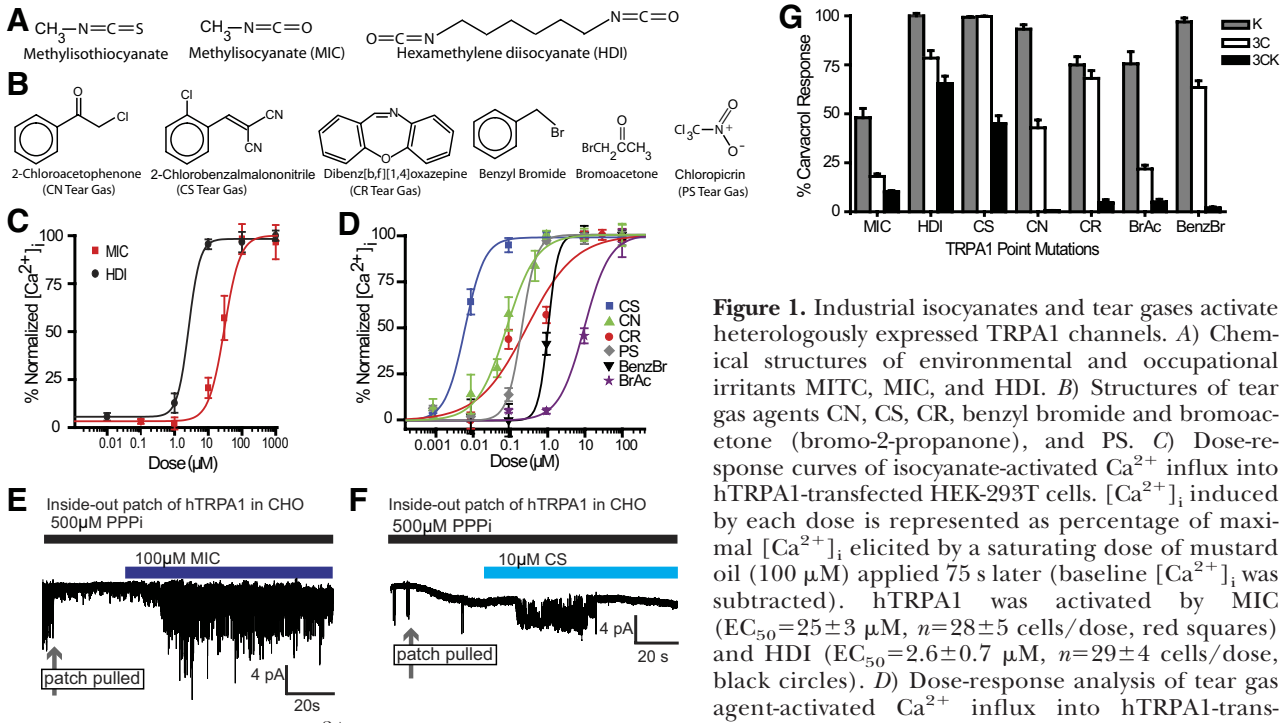

Figure 1. Industrial isocyanates and tear gases activate heterologously expressed TRPA1 channels. *A*) Chemical structures of environmental and occupational irritants MITC, MIC, and HDI. *B*) Structures of tear gas agents CN, CS, CR, benzyl bromide and bromoacetone (bromo-2-propanone), and PS. *C*) Dose-response curves of isocyanate-activated Ca^{2+} influx into hTRPA1-transfected HEK-293T cells. $[Ca^{2+}]_i$ induced by each dose is represented as percentage of maximal $[Ca^{2+}]_i$ elicited by a saturating dose of mustard oil (100 μM) applied 75 s later (baseline $[Ca^{2+}]_i$ was subtracted). hTRPA1 was activated by MIC (EC_{50}=25±3 μM, n=28±5 cells/dose, red squares) and HDI (EC_{50}=2.6±0.7 μM, n=29±4 cells/dose, black circles). *D*) Dose-response analysis of tear gas agent-activated Ca^{2+} influx into hTRPA1-transfected HEK-293T cells. $[Ca^{2+}]_i$ represented as in *A*. hTRPA1 was activated by CN (EC_{50}=91±12 nM, n=49±1 cells/dose, green triangles), CS (EC_{50}=7±1 nM, n=52±5, blue squares), CR (EC_{50}=308±150 nM, n=70±9, red circles), PS (EC_{50}=215±62 nM, n=32±4, gray diamonds), benzyl bromide (BenzBr, EC_{50}=12±1 μM, n=66±17, black triangles) and bromoacetone (BrAc, EC_{50}=1.1±0.3 μM, n=78±14 cells/dose, purple stars). *E*) Application of MIC (100 μM, purple bar) induces an increase of single-channel openings of an excised patch in the inside-out configuration from hTRPA1-overexpressing CHO cells. Voltage was held at −40 mV; bath solution contained 0.5 mM PPP_i and was devoid of Ca^{2+} (10 mM EGTA). *F*) Application of CS (10 μM, blue bar) induces an increase of single-channel openings in an excised patch in the inside-out configuration from hTRPA1-overexpressing CHO cells. Conditions were same as for *E*. *G*) Responses of hTRPA1 mutant-expressing HEK-293T cells to 100 μM MIC, 100 μM HDI, 100 μM CS, 100 μM CN, 300 μM CR, 100 μM bromoacetone (BrAc), and 100 μM benzyl bromide (BenzBr). Increase in $[Ca^{2+}]_i$ is displayed as percentage of $[Ca^{2+}]_i$ activated by a saturating dose of carvacrol (300 μM). Mutants tested are coded as follows: K (gray) = K708 (MIC, n=84; HDI, n=53; CS, n=157; CN, n=108; CR, n=154; BrAc, n=44; BenzBr n=64 cells); 3C (white) = C619, C639, and C663 combined (MIC, n=34; HDI, n=47; CS, n=96; CN, n=56; CR, n=90; BrAc, n=25; BenzBr, n=40 cells); 3CK (black) = C619, C639, and C663; and K708 combined (MIC, n=30; HDI, n=123; CS, n=129; CN, n=18; CR, n=17; BrAc, n=38; BenzBr, n=41).

pain with antiinflammatory drugs and general and local anesthetics, and stabilization of the airways with bronchodilators (13). Although these procedures are helpful, the additional use of pharmacological agents to block the specific targets of isocyanates and tear gases would allow a more efficient treatment to alleviate acute irritation and pain and to prevent the development of chronic health effects.

Although immunological pathways are thought to mediate the allergic sensitization to isocyanates in the airways, studies in animal models point to a role of peripheral sensory C-fibers in their acute noxious effects and in exposure-induced airway hyperreactivity (14–19). In guinea pigs, isocyanates stimulate the release of neuropeptides from capsaicin-sensitive (C-fiber) airway nerve endings, leading to constriction of isolated bronchial segments (20, 21). Similar to the airways, the cornea of the eye is densely innervated by peripheral sensory nerve fibers. A majority of these fibers are trigeminal chemosensory C-fibers that trigger the lacrimation reflex after exposure to a noxious chemical stimulus (22). In addition to lacrimation, activation of corneal C-fibers induces ocular pain and blepharospasm, both of which are symptoms associated with tear gas exposures (23). Ocular pretreatment with local anesthetics abolishes the tear gas-induced lacrimation reflex, suggesting that these agents target corneal chemosensory nerve endings (22).

Peripheral sensory neurons express a large number of excitatory or sensitizing chemosensory receptors, including members of the transient receptor potential (TRP) ion channel family (24–26). Natural products activating the sensory neuronal TRP channels, transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1), induce effects similar to those of industrial isocyanates and tear gases. For example, the key ingredient of pepper spray, capsaicin, is a specific agonist of TRPV1 (27, 28). TRPA1 is the receptor for mustard oil (allyl isothiocyanate), the pungent ingredient in mustard, for allicin and diallyl disulfide, the lacrimatory principles in garlic and onions, and pungent natural dialdehyde sesquiterpenes (29–33). In addition to natural products, TRPA1 is also activated by industrial and environmental elec-

trophilic and oxidizing chemicals (34–36). For example, TRPA1 is activated by hypochlorite, the reactive mediator of the potent irritant gas chlorine, and is crucial for oxidant-induced respiratory depression and nocifensive behavior in mice (36–38). The role of TRPA1 as a major chemical irritant sensor in airway sensory neurons was further corroborated by experiments showing its essential requirement for cigarette smoke extract-induced neurogenic inflammation in mice and guinea pigs and by findings describing its interaction with endogenous reactive mediators enriched during airway inflammation (39–43).

Recent studies by us and others have shown that TRPA1 is activated by chemical tear gas agents *in vitro*, including acrolein, CN, CS, and CR (34, 44). Because these chemicals are highly reactive and may induce nonspecific tissue damage, it is questionable whether all of them selectively and potently target TRPA1 *in vivo*. Reactive agents may be inactivated before reaching sensory neuronal targets or activate neurons indirectly through factors released from damaged tissue. For example, adenosine or ATP released from airway tissue damaged by inhalation of organic chemical or acidic fumes has been shown to activate sensory neurons through interaction with purinergic receptors (45). Thus, detailed whole animal physiological, pharmacological, and behavioral studies are required for validation of TRPA1 as a specific target for any given chemical *in vivo*.

The molecular targets for industrial isocyanates in sensory neurons are unknown. Isocyanates are highly electrophilic compounds chemically related to isothiocyanates such as mustard oil. Methylisothiocyanate (MITC), the isothiocyanate analog of MIC, is a widely used soil fumigant that frequently causes irritation and occupational injuries in agricultural workers (Fig. 1*A*) (3, 4). In comparison with mustard oil, MITC is only a weak agonist of TRPA1 *in vitro* (29). Evidence suggests that activation of TRPA1 by reactive chemicals such as isocyanates and isothiocyanates occurs through covalent modification of cytosolic amino acid residues in the N terminus of the ion channel protein (46, 47). Intriguingly, ruthenium red, a blocker of TRPA1 and other TRP channels, inhibits isocyanate-induced contraction of isolated guinea pig bronchi (21). Thus, activation of sensory neuronal TRP ion channels may contribute to the immediate noxious effects of isocyanate exposures *in vivo*.

The purpose of our present study was to identify and characterize potential targets for industrial isocyanates and for tear gas agents in peripheral sensory neurons and to examine their roles in ocular and facial irritant sensation *in vivo*. Our findings suggest that TRPA1 is the major mediator of sensory neuronal activation by isocyanates and tear gas agents, both *in vitro* and *in vivo*. Newly developed TRPA1 antagonists selectively block neuronal activation by these agents, providing a promising lead for future therapies of chemical exposures.

MATERIALS AND METHODS

Animals

Mice were housed at an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility in standard environmental conditions (12-h light/dark cycle and ~23°C). All animal procedures were approved by the Yale Institutional Animal Care and Use Committee. Animals were identically matched for age (12–22 wk) and sex, and the experimenter was masked to the genotype. *Trpa1*$^{-/-}$ mice were a gift from David Julius (University of California, San Francisco, CA, USA) and were genotyped as described (33). C57 mice were purchased commercially (Charles River Breeding Laboratories, Inc., Wilmington, MA, USA). In certain experiments, 200-μl i.p. injections of 0, 1, 2, or 6 mg of HC-030031 dissolved in 0.5% methylcellulose (Methocel; Fluka AG, Buchs, Switzerland) were administered to mice.

Cell culture

Adult mouse dorsal root ganglia and trigeminal ganglia were dissected and dissociated by a 1-h incubation in 0.28 Wünsch units/ml Liberase Blendzyme 1 (Roche Diagnostics, Mannheim, Germany), followed by washes with Hanks' buffered saline, trituration, and straining (70 μM; Falcon; BD Biosciences Discovery Labware, Bedford, MA, USA). Trigeminal ganglia were further purified using centrifugation over a Percoll gradient (GE Healthcare, Chalfont St. Giles, UK). Neurons were cultured in Neurobasal-A medium (Invitrogen, Carlsbad, CA, USA) with B-27 supplement, 0.5 mM glutamine, and 50 ng/ml nerve growth factor (Merck Biosciences, Darmstadt, Germany) on 8-well chambered coverglass or 35-mm dishes (Nunc, Roskilde, Denmark) coated with polylysine (Sigma-Aldrich Corp., St. Louis, MO, USA) and laminin (Invitrogen). Human embryonic kidney (HEK-293T) and Chinese hamster ovary (CHO) cells for Ca^{2+} imaging and electrophysiology were cultured and transfected with human and mouse TRPA1, mutant TRPA1, rat TRPV1, or empty vector (pcDNA3) cDNAs as described previously (29, 33).

Chemicals and solutions

If not otherwise indicated, chemicals were purchased from Sigma-Aldrich Corp. Whole-cell electrophysiological and Ca^{2+}-imaging experiments were performed in modified standard Ringer's bath solution: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES-NaOH, and 5 mM glucose (pH 7.3, 315–320 mosM). Pipette and chip solutions for whole-cell intracellular application contained 75 mM CsCl, 70 mM CsF, 2 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES-CsOH (pH 7.3, 315–320 mosM). Pipette and bath solutions for single-channel electrophysiological recordings contained solutions identical to the standard Ringer's bath solution with the exception of being Ca^{2+}-free and containing 10 mM EGTA. Solutions for recordings in the inside-out configuration contained 0.5 mM sodium tripolyphosphate (PPPi) (Acros Organics, Fairlawn, NJ, USA). In certain cell-attached recordings, solutions contained 2 mM $CaCl_2$ and did not contain EGTA and PPPi. Isocyanate solutions of MDI (Chem Service Inc., West Chester, PA, USA) and HDI and tear gas solutions of CN (Scientific Exchange, Inc., Center Ossipee, NH, USA) and CR (Key Organics Ltd., Camelford, UK) were initially dissolved in dimethyl sulfoxide (DMSO) at 40 mM. Ionomycin (4 mM; MP Biomedicals, Solon, OH, USA), capsaicin (100 mM), and 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphorylcholine (ET-18-OCH3) (20 mM) were dissolved in ethanol, and ruthenium red (100 mM;

Latoxan, Valence, France) was dissolved in water. Stock solutions were diluted to their final concentration in appropriate solution for applications. For eye applications, HDI, CN, and CS were dissolved in 75% DMSO/PBS to 100 mM. A freezing point osmometer (Advanced Instruments, Norwood, MA, USA) was used to measure the osmolarity of all solutions. The TRPA1 antagonists 4-(4-chlorophenyl)-3-methylbut-3-en-2-oxime (AP-18) (10 mM; Maybridge, Trevillett, UK) and 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7*H*-purin-7-yl)-*N*-(4-isopropylphenyl)acetamide (HC-030031) (20 mM; Hydra Bioscience, Cambridge, MA, USA) were dissolved in DMSO. For i.p. injections, 5, 10, and 30 mg/ml HC-030031 was suspended in 0.5% methylcellulose (Methocel).

Ca^{2+} imaging and electrophysiology

Cultured neurons and HEK-293T cells were loaded in modified Ringer's solution with 10 μM Fura-2-AM (Calbiochem, San Diego, CA, USA) and 0.02% Pluronic F127 (BASF, Mount Olive, NJ, USA) for 1 h and subsequently washed and imaged in glucose-free modified Ringer's solution. Fura-2-AM emission ratios were obtained with alternating 0.100-ms exposures at 340 and 380 nm from a Polychrome V monochromator (Till Photonics, Gräfelfing, Germany) on a microscope (IX51; Olympus, Center Valley, PA, USA), captured with a PCO camera (Sensicam QE; Cooke, Auburn Hills, MI, USA), and analyzed with Imaging Workbench 6 software (Indec; Santa Clara, CA, USA). Intracellular calcium ($[Ca^{2+}]_i$) concentrations were derived from the F_{340}/F_{380} ratio adjusted by the K_d of Fura-2-AM (238 nM) and the F_{380} and ratiometric data at minimum and maximum $[Ca^{2+}]_i$ (48–50). The latter was determined by incubation in 10 μM ionomycin Ringer's solution with 0 Ca^{2+} 10 mM EGTA or 25 mM Ca^{2+} (90 mM NaCl to compensate for a final osmolarity of 350 mosM). Ratiometric images were generated using ImageJ software (http://rsbweb.nih.gov/ij/).

Whole-cell configuration patch-clamp experiments were performed at ~25°C with borosilicate glass pipettes (World Precision Instruments, Sarasota, FL, USA) for neurons and a planar patch-clamp system for HEK-293T cells (NPC-1; Nanion, Munich, Germany). High-resolution currents were filtered at 2.3 kHz and digitized at 100-μs intervals using an EPC-10 amplifier (HEKA, Lambrecht, Germany) and Pulsemaster acquisition software (HEKA). Voltage ramps of −100 to +100 mV or −80 to +80 mV were applied over 100 ms at every 0.5 Hz from a holding potential of 0 mV as described previously (51). A liquid junction potential of 6.7 mV (JPCalc software; Axon Instruments, MA, USA) and capacitance were compensated for by the amplifier system.

Single-channel patch-clamp experiments were performed in the cell-attached or inside-out configurations on CHO cells at ~25°C with wax-coated borosilicate glass pipettes (World Precision Instruments). High-resolution currents were filtered at 3 kHz and digitized at 20-μs intervals using an EPC-10 amplifier and Pulsemaster acquisition software (HEKA).

Analysis of nocifensive responses

The nocifensive responses to intraocular instillation of 10 μl of 100 or 200 mM HDI, 100 mM CN, or 100 mM CS into the right eye or vehicle control (70% DMSO/PBS) into the left eye of *Trpa1*$^{-/-}$ and *Trpa1*$^{+/+}$ mice were video recorded (DCR-SR80; Sony, New York, NY, USA) in a clear Plexiglass cylinder (5-inch inside diameter) for 2 or 3 min. At the conclusion of every test, the treated eye was irrigated with PBS. Mice responded to HDI or tear gas agent application by lowering and subsequent pushing or rubbing of the facial area on the floor of the behavioral recording chamber; responses were individually counted. Nearly identical experiments were conducted on C57 mice after a 200-μl i.p. injection of 0.5% vehicle control (100 mM HDI, 100 mM CN, or 100 mM CS intraocular instillation into the right eye) and ~1 h after a 200-μl i.p. injection of the TRPA1 antagonist HC-030031 (1 or 6 mg) (100 mM HDI, 100 mM CN, or 100 mM CS into the right eye).

Nocifensive responses in the paw were also examined by 25-μl intraplantar injections using a 30-gauge needle. For experiments in *Trpa1*$^{-/-}$ and *Trpa1*$^{+/+}$ mice, vehicle was injected into the left paw and then ~1 h later either CN (2 mM in 5% DMSO/PBS) or bromoacetone (4 mM in PBS) was injected in the right paw. For experiments using HC-030031 on C57 mice, HDI (6 mM in 5% DMSO/PBS) or CN (4 mM in 5% DMSO/PBS) was injected into the right paw after a 200-μl i.p. injection of 0.5% vehicle control, and ~1 h after mice received a 2-mg i.p. injection of HC-030031 (200 μl), 6 mM HDI or 4 mM CN was injected into the left paw. Videorecorded responses (licking, lifting, and flicking of the injected paw) in a Plexiglas cylinder for 3–5 min were visualized and quantified by slowing the video frame speed using Microsoft Windows Media Player software (Microsoft, Redmond, WA, USA). The more hydrophobic agents were not used, because they were insoluble in 5% DMSO.

Statistics

Statistical analysis was performed, and graphical displays of both electrophysiological and Ca^{2+}-imaging data were made using IGOR Pro (Wavemetrics, Lake Oswego, OR, USA) or ORIGIN (OriginLab, Northampton, MA, USA). Statistical errors are SE unless indicated otherwise.

An independent two-sample Student's *t* test was performed between mice lacking a functional *Trpa1* gene (*Trpa1*$^{-/-}$) and wild-type littermates (*Trpa1*$^{+/+}$) on the total quantity of paw licking, lifting, and flicking (paw pain response, n=8/group for CN and n=6/group for bromoacetone) and the "facial pain" of stroking the orbitofacial area in response to isocyanate or tear gases (CS and HDI n=6 *Trpa1*$^{+/+}$, n=7 *Trpa1*$^{-/-}$; n=6/group for CN). Differences were seen in the paw response to CN (P=0.023) and bromoacetone (P=0.045). Differences were seen in the facial pain response to CN (P=0.001), CS (P=0.001), and HDI (P=0.008).

Dependent (repeated-measure) Student's *t* tests were performed on the mouse facial pain and paw pain responses to isocyanate or tear gases after vehicle control injection compared with the responses ~1 h after the mice were injected with 6 mg of HC-030031 (n=6/group for CS and HDI; n=9 for CN), 1 mg of HC-030031 (n=6/group), or 2 mg of HC-030031 (n=6/group). Differences were seen in facial pain with the 1-mg HC-030031 treatment to applications of CN (P=0.004), CS (P=0.04), and HDI (P=0.01) and with the 6-mg HC-030031 treatment to applications of CN (P=0.0), CS (P=0.005), and HDI (P=0.029). Differences in paw pain were observed after the 2-mg HC-030031 treatment to intraplantar injections of CN (P=0.005) and HDI (P=0.015).

RESULTS

TRPA1 is activated by industrial isocyanates and all major tear gas agents *in vitro*

We used fluorescent $[Ca^{2+}]$ imaging to examine the effects of 2 major industrial isocyanates (Fig. 1*A*) and 6 different tear gas agents (Fig. 1*B*) on 2 members of the TRP ion channel family, TRPV1, the capsaicin receptor,

and TRPA1, the mustard oil receptor, expressed in HEK-293T cells. TRPA1 was strongly activated by MIC, HDI, and all of the tear gas agents tested (CN, CS, CR, PS, bromoacetone, and benzyl bromide). Dose-response analysis revealed that the isocyanates MIC (EC_{50}=25±3 μM, n=28±5 cells/dose) and HDI (EC_{50}=2.6±0.7 μM, n=29±4 cells/dose) activated TRPA1 with a potency comparable to that of the chemically similar mustard oil (allyl isothiocyanate) (Fig. 1*C*). In our hands, CS was the most potent activator of human TRPA1 channels, with half-maximal activation occurring at EC_{50} CS = 7 ± 1 nM (n=52±5 cells/dose) and was 3 orders of magnitude more potent than mustard oil. CN, CR, and PS were also highly potent, with half-maximal activation of human TRPA1 (hTRPA1) at EC_{50} CN = 91 ± 12 nM (n=49±1 cells/dose), EC_{50} CR = 308 ± 150 nM (n=70±9 cells/dose), and EC_{50} PS = 308 ± 150 nM (n=32±4 cells/dose) (Fig. 1*D*). The tear gas agents benzyl bromide (EC_{50}=12.0±0.6 μM, n=66±17 cells/dose) and bromoacetone (EC_{50}=1.1±1.1 μM, n=78±14 cells/dose) also activated hTRPA1. At saturating doses of the noxious chemical activation of hTRPA1, neither rat TRPV1- nor empty vector (pcDNA3)-transfected HEK-293T cells responded (Supplemental Fig. 1*A, B*). Only HDI and benzyl bromide induced minor TRPV1 activity after significant delays following irritant application (Supplemental Fig. 1*B*).

Recent studies support the idea that reactive irritants activate TRPA1 through covalent modification of cysteine and lysine residues within the cytosolic N terminus of the channel protein (46, 47). Whereas isocyanates and some tear gas agents can undergo electrophilic chemical reactions, CN, CS, and CR also share structural similarities with nonreactive TRPA1 agonists, including terpenes such as carvacrol or thymol (44, 52, 53). Chemical agents may also activate TRPA1 indirectly, through stimulation of phospholipase C (PLC)-coupled receptor pathways and subsequent release of Ca^{2+} from intracellular stores or through other Ca^{2+}-mobilizing pathways (29, 30, 54–56). To examine the requirement for Ca^{2+} or other cytosolic factors, we performed inside-out patch-clamp recordings of hTRPA1 channels expressed in CHO-K1 cells in the absence of Ca^{2+} on both sides of the membrane. In this configuration, PLC- and any other second messenger-dependent pathways are disrupted. Sodium triphosphate (0.5 μM), an essential intracellular cofactor for TRPA1 activation, was included in the bath solution (57). Application of 100 μM MIC or 10 μM CS specifically induced a large increase in single-channel openings (124±3 pS for MIC and 120±3 pS for CS at −40 mV; 3 patches/agent), similar to TRPA1 single conductances recorded by others in the absence of Ca^{2+} (Fig. 1*E, F*; Supplemental Fig. 2*A, B*) (58). These results suggest that isocyanates and tear gas agents activate TRPA1 in a membrane-delimited fashion that does not require increases in cytosolic Ca^{2+} or activation of second messenger pathways. hTRPA1 single channels were also activated in the cell-attached configuration, indicating that the chemical activator needs to traverse the plasma membrane to activate the ion channels positioned under the patch electrode (Supplemental Fig. 2*D, E*). The open channel current-voltage relationship of HDI-activated channels in the cell-attached configuration was linear in the absence of Ca^{2+} (single channel conductance: 127±4 pS at −40 mV) but outwardly rectifying in the presence 2 mM Ca^{2+} (51±2 pS at −40 mV) (Supplemental Fig. 2*D, E*).

We next examined whether isocyanates and tear gas agents would require putative covalent acceptor sites in hTRPA1 for channel activation (46, 47). We examined three different mutant channels in which critical reactive sites (C619, C639, C663, and K708) were replaced by inert residues. In the first mutant (K) K708 was replaced. A second mutant (3C) had mutations in all three cysteine residues, and a third mutant (3CK) had mutations in all four sites. In previous studies these mutations dramatically reduced the potencies and efficacies of electrophiles and oxidants to activate TRPA1 (33, 36, 46, 47). As a positive control, we used the TRPA1 agonist carvacrol, a pungent nonreactive terpene, which does not activate TRPA1 by covalent binding. Whereas the lysine mutant was activated by all agents (MIC, n=84; HDI, n=53; CS, n=157; CN, n=108; CR, n=154; bromoacetone, n=44; and benzyl bromide, n=64), mutant 3C showed significantly reduced responses to MIC (n=34), CN (n=56) and CR (n=90), and bromoacetone (n=25) but did not greatly affect the efficacy of HDI (n=47), CS (n=96), or benzyl bromide (n=40). CS, the most potent tear gas agent also showed significant activity on the 3CK mutant (n=129), as did the isocyanate HDI (n=123), indicating that these agents may require additional reactive sites for their activity or activate TRPA1 through a different mechanism. In contrast, the activities of the other tested chemicals were dramatically reduced or eliminated (MIC, n=30; CN, n=18; CR, n=17; bromoacetone, n=38; and benzyl bromide, n=41) (Fig. 1*G*).

Cellular responses of native sensory neurons to industrial isocyanates have not been reported. We therefore used fluorescent Ca^{2+} imaging to investigate the effects of MIC and HDI on dissociated murine trigeminal ganglion (TG) and dorsal root ganglion (DRG) neurons. Fibers derived from the TG innervate the eyes, facial skin, and upper airways that were the initial contact sites of exposure in patients during the Bhopal incident. DRG neurons innervate parts of the lower airways affected after inhalation of the toxicant. We observed that MIC (100 μM) and HDI (100 μM) induced a rapid increase in $[Ca^{2+}]_i$ in a subset of capsaicin-sensitive TG and DRG neurons, overlapping with the mustard oil-sensitive neuronal population (**Fig. 2*A, B*;** Supplemental Fig. 1*E*).

Responsiveness of native sensory neurons to the two most widely used tear gas agents, CS and CN, has not been described. CR was recently reported to activate Ca^{2+} influx into cultured DRG neurons (44). However, although implying that TRPA1 was a neuronal target for CR, this study did not use any specific pharmaco-

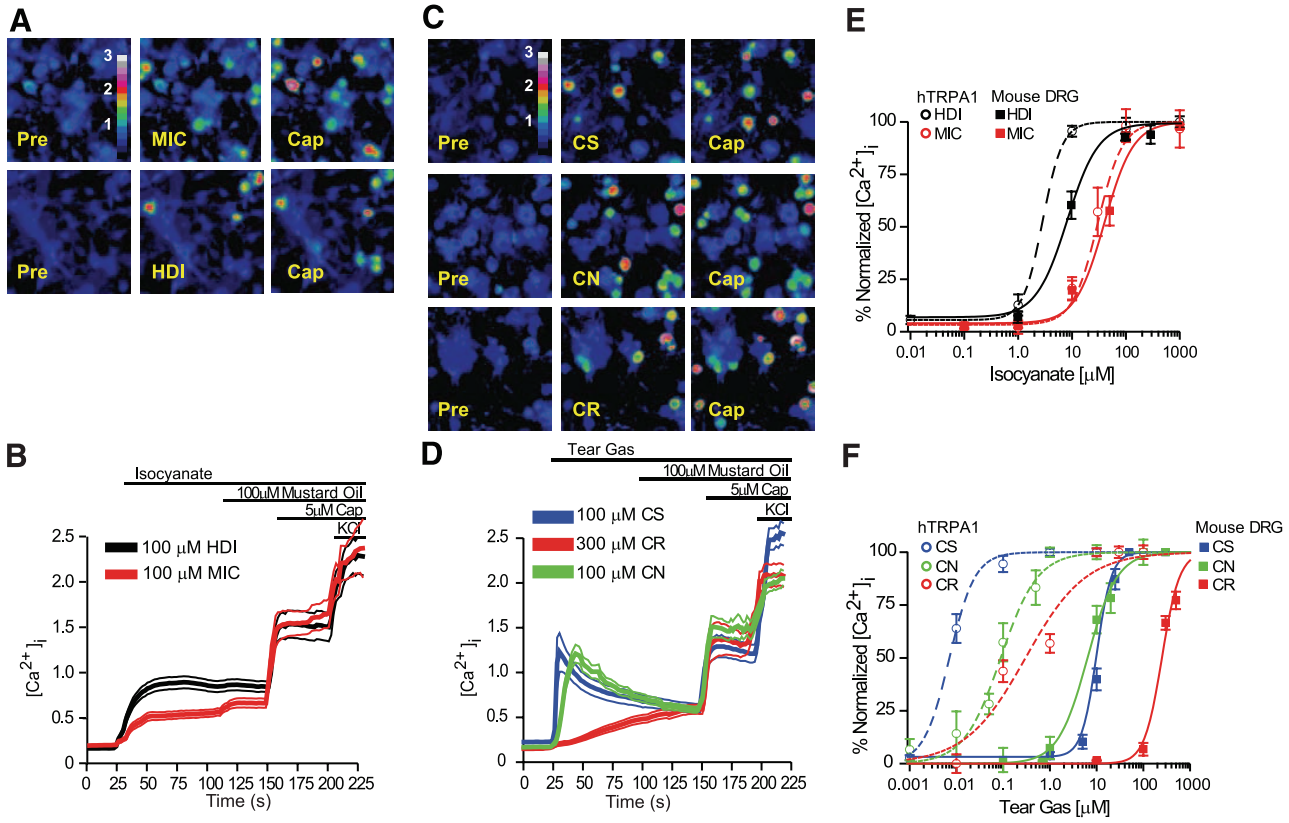

Figure 2. Industrial isocyanates and tear gas agents activate native TRPA1 channels in cultured sensory neurons. *A*) Industrial isocyanates induced Ca^{2+} influx into cultured mouse DRG neurons, as measured by fluorescent Fura-2 imaging. Neurons are shown before activation (Pre, left column), 70 s after challenge (middle column) with MIC (100 μM, top row) or HDI (100 μM, bottom row), and after application of 5 μM capsaicin (Cap, right column) after 50 s. Pseudocolors denote 0–3 μM $[Ca^{2+}]_i$. Original view, ×10. *B*) Average $[Ca^{2+}]_i$ of mouse DRG neurons (thick lines) with an application of MIC (100 μM, n=168 neurons from 2 mice, black line) or HDI (100 μM, n=270 from 2 mice, red line), followed by 100 μM mustard oil, 5 μM capsaicin (Cap), and 65 mM KCl. Thin lines represent SE. *C*) Tear gas agent-induced Ca^{2+} influx into cultured murine DRG neurons, as measured by fluorescent Fura-2 imaging. Neurons are shown before activation (Pre, left column), 70 s after challenge (middle column) with CS (100 μM, top row) or CN (100 μM, middle row) or CR (300 μM, bottom row) and after application of 5 μM capsaicin (Cap, right column) after 50 s. Pseudocolors denote 0–3 μM $[Ca^{2+}]_i$. Original view, ×10. *D*) Average $[Ca^{2+}]_i$ of mouse DRG neurons (thick lines) with an application of CS (100 μM, blue line, n=161 neurons from 2 mice), CN (100 μM, green line, n=335 from 5 mice), or CR (300 μM, red line, n=137 from 2 mice), followed by 100 μM mustard oil, 5 μM capsaicin (Cap), and 65 mM KCl. Thin lines represent SE. *E*) Dose-response curves of isocyanate-activated Ca^{2+}-influx into mouse DRG neurons are similar to hTRPA1-transfected HEK-293T cells. $[Ca^{2+}]_i$ induced by each dose is represented as percentage of maximal $[Ca^{2+}]_i$ elicited by a saturating dose of mustard oil (100 μM) applied 75 s later (baseline $[Ca^{2+}]_i$ was subtracted). Mustard oil-sensitive mouse DRG neurons were activated by MIC (EC_{50}=36±7 μM, n=30±6 neurons/dose, solid black squares) and HDI (EC_{50}=8.4±1.4 μM, n=39±12 /dose, solid red squares). Dashed lines and open circles represent hTRPA1-transfected HEK-293T cells, as shown in Fig. 1*C*. *F*) Dose-response curves of tear gas agent-activated Ca^{2+}-influx into mouse DRG neurons are right-shifted compared with responses in hTRPA1-transfected HEK293T cells. $[Ca^{2+}]_i$ represented as in *E*. Mustard oil-sensitive mouse DRG neurons were activated by CS (EC_{50}=12.1±0.3 μM, n=41±9 neurons/dose, solid blue squares), CN (EC_{50} =6±1 μM, n=23±5 /dose, solid green squares), and CR (EC_{50}= 246±27 μM, n=37±16 /dose, solid red squares). Dashed lines with open circles represent dose-response curves of Ca^{2+}-influx into hTRPA1-transfected HEK-293T cells shown in Fig. 1*D*.

logical, genetic, or *in vivo* approaches to substantiate this point. We found that CS, CN, bromoacetone, and benzyl bromide (100 μM each) rapidly induced Ca^{2+} influx into a subset of DRG neurons (Fig. 2*C, D, F*; Supplemental Fig. 1*C*). Exposure to CS, CN, bromoacetone, and benzyl bromide eliminated the neuronal sensitivity to subsequent application of mustard oil. CR (300 μM) only slowly induced neuronal activity and did not completely prohibit further neuronal activation by mustard oil (Fig. 2*D*). CS and CN also induced Ca^{2+} influx into TG neurons (Supplemental Fig. 1*F*).

Similar to previously characterized TRPA1 agonists such as mustard oil or acrolein, the isocyanates have very similar potencies in mustard oil-sensitive DRG neurons (EC_{50} MIC=36±7 μM, n=30±6 neurons/dose) and (EC_{50} HDI=8.4±1.4 μM, n=39±12 neurons/dose) and in hTRPA1-transfected cells (EC_{50} MIC=25±3 μM) and HDI (EC_{50} HDI=2.6±0.7 μM) (Fig. 2*E*). Most surprisingly, we found the tear gas agents CS and CR to be ~1000-fold less potent and CN to be 100-fold less potent for activating Ca^{2+} influx into native neurons (EC_{50} CS=12.1±0.3 μM, n=41±9 neu-

rons/dose; EC_{50} CN=6±1 μM, n=23±5 neurons/dose; and EC_{50} CR=246±27 μM, n=37±16 neurons/dose) compared with heterologous cells expressing hTRPA1 (EC_{50} CS=7±1 nM, EC_{50} CN=91±12 nM, and EC_{50} CR=308±150 nM) (Fig. 2*F*) or mouse TRPA1 (EC_{50} CN=66±14 nM) (Supplemental Fig. 1*F*).

The large divergence of tear gas agent potencies between heterologous cells expressing TRPA1 and primary neurons suggests that either native TRPA1 channels have different pharmacological properties or that alternative targets may be involved in neuronal responses to these agents. To further examine the neuronal response to tear gas agents we performed patch-clamp electrophysiological recordings of primary neurons in the whole-cell configuration. CN (100 μM) induced sizable, slightly outwardly rectifying membrane currents in 4 of 16 recorded neurons, which were efficiently blocked by ruthenium red, a pore blocker of TRPA1 and other TRP ion channels (**Fig. 3*A*, *B***). The percentage of responsive neurons, the size, and the current-voltage (I-V) relationship of the CN-induced currents were similar to neuronal TRPA1 currents we recorded in previous studies using the TRPA1 agonists sodium hypochlorite and isovelleral (33, 36). Furthermore, the CN-induced neuronal currents were remarkably similar in their relationship to voltage to CN-induced currents in hTRPA1-expressing HEK-293T cells (Fig. 3*C*). Compared with neuronal currents, TRPA1 currents in the heterologous HEK-293T cells were larger and desensitized rapidly, as characterized by us and others with a variety of agonists (n=4) (Fig. 3*D*) (36).

Genetic deletion of TRPA1 or pharmacological blockade with TRPA1 antagonists renders sensory neurons insensitive to isocyanates and tear gas agents

Our results gathered from cultured primary neurons and heterologous cells suggest that industrial isocyanates and tear gas agents excite sensory neurons through activation of TRPA1. However, concentrations of tear gas agents required to induce Ca^{2+} influx into cultured sensory neurons were >100-fold higher than required for activation of cloned mouse and human TRPA1 channels expressed in heterologous cells. It remained a possibility that isocyanates and tear gas agents activated alternative targets in sensory neurons, through direct interactions with other Ca^{2+}-permeable ion channels with relatively similar electrophysiological profiles, or indirectly, through activation of signal transduction cascades involving PLC. PLC pathways have been shown to activate or sensitize TRPA1 and many other Ca^{2+}-permeable TRP ion channels (26, 59). To investigate the potential involvement of PLC pathways in the neuronal response to isocyanates and tear gases, we performed Ca^{2+}-imaging experiments in the presence of ET-18-OCH_3, a PLC inhibitor used in a previous study to inhibit activation of TRPA1 through PLC-coupled protease-activated receptors in sensory neurons (56). ET-18-OCH_3 (4 μM) did not diminish neuronal Ca^{2+} influx activated by any of the noxious agents applied (Supplemental Fig. 1*D*).

To examine the requirement for TRPA1 in sensory neuronal responses to isocyanates and tear gas agents, we studied the responses of sensory neurons dissociated from TRPA1-deficient mice. When superfused with MIC (n=217 neurons from 2 mice), HDI (n=204 neurons from 2 mice), CS (n=229 neurons from 2 mice), CN (n=270 neurons from 5 mice), or CR (n=108 neurons), TRPA1-deficient neurons failed to respond with an increase in $[Ca^{2+}]_i$. These neurons responded normally to capsaicin, used as a control stimulus (Fig. 3*A–D*).

Recently, the structures and efficacies of two newly developed TRPA1 antagonists were reported (35, 60). These antagonists, HC-030031 and AP-18, blocked the activation of TRPA1 by mustard oil and other reactive

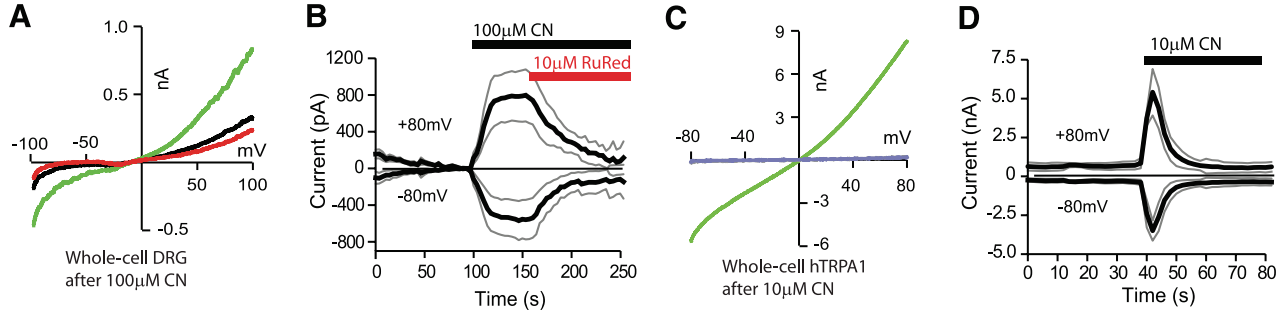

Figure 3. CN induces TRPA1-like currents in mouse DRG neurons. *A)* TRPA1-like current-voltage curves of a representative mouse DRG neuron before activation (black trace), activation by 100 μM CN (green trace), and inhibition by ruthenium red (10 μM, red trace) in whole-cell configuration. $V_{holding}$ = 0 mV to minimize voltage-gated channels. Currents were measured with a voltage ramp from −100 to +100 mV over 100 ms at 0.5-Hz intervals. Intracellular Cs-based solution with 10 mM EGTA was used. *B)* Average native TRPA1-like currents at −80 and +80 mV in mouse DRG neurons superfused with 100 μM CN (black bar), followed by ruthenium red (RuRed; 10 μM) as described for Fig. 1*B* (n=4 of 16 neurons). Baseline current was subtracted for each trace. *C)* hTRPA1 current-voltage curves before activation (black trace), at maximal activation by 10 μM CN (green trace), and after inactivation phase (blue trace) in whole-cell configuration. Currents were measured with a voltage ramp from −80 to +80 mV over 100 ms at 0.5 Hz intervals, $V_{holding}$= 0 mV. Intracellular Cs-based solution with 10 mM EGTA was used. *D)* Averaged TRPA1 currents at −80 and +80 mV in hTRPA1-transfected HEK-293T cells superfused with 10 μM CN (black bar) as described for Fig. 1*C* (n=4).

chemical stimuli *in vitro*. We examined the effects of these antagonists on CS-, CN- and CR-induced activation of hTRPA1 expressed in HEK-293T cells. Both HC-030031 and AP-18, used at a concentration of 25 μM, efficiently blocked the activation of hTRPA1 by all three tear gas agents (Supplemental Fig. 3*A*). The antagonist HC-030031 effectively blocked native TRPA1 responses to 10 μM HDI (IC_{50}=74±3 μM, n=31±4), CN (IC_{50}=884±23 nM, n=25±5), and CS (IC_{50}=4.5±0.4 μM, n=26±6) in cultured sensory neurons dissociated from wild-type mice (**Fig. 4*D***; Supplemental Fig. 3*B, C*). These neurons responded normally to a saturating dose of capsaicin, used as a control stimulus.

Taken together, our results show that TRPA1 is the sole target of industrial isocyanates and tear gas agents in sensory neurons, allowing influx of Ca^{2+} and neuronal excitation. Furthermore, we show that TRPA1 antagonists completely block neuronal activity in response to isocyanates or tear gas agents. This finding suggests that TRPA1 antagonists may prevent and alleviate the noxious effects of isocyanates and tear gas agents *in vivo*.

TRPA1 antagonists effectively block the noxious effects of isocyanates and tear gas agents in vivo

Human exposure to airborne industrial isocyanates and tear gases results in immediate extreme ocular and facial pain, as well as in airway irritation, mucus secretion, and obstruction. Our data suggest that these effects are triggered by activation of TRPA1 channels in trigeminal sensory neurons. However, it is unclear whether isocyanates and tear gas agents interact specifically with TRPA1 *in vivo* or whether these highly reactive chemicals activate sensory neurons indirectly through factors released during tissue damage. We

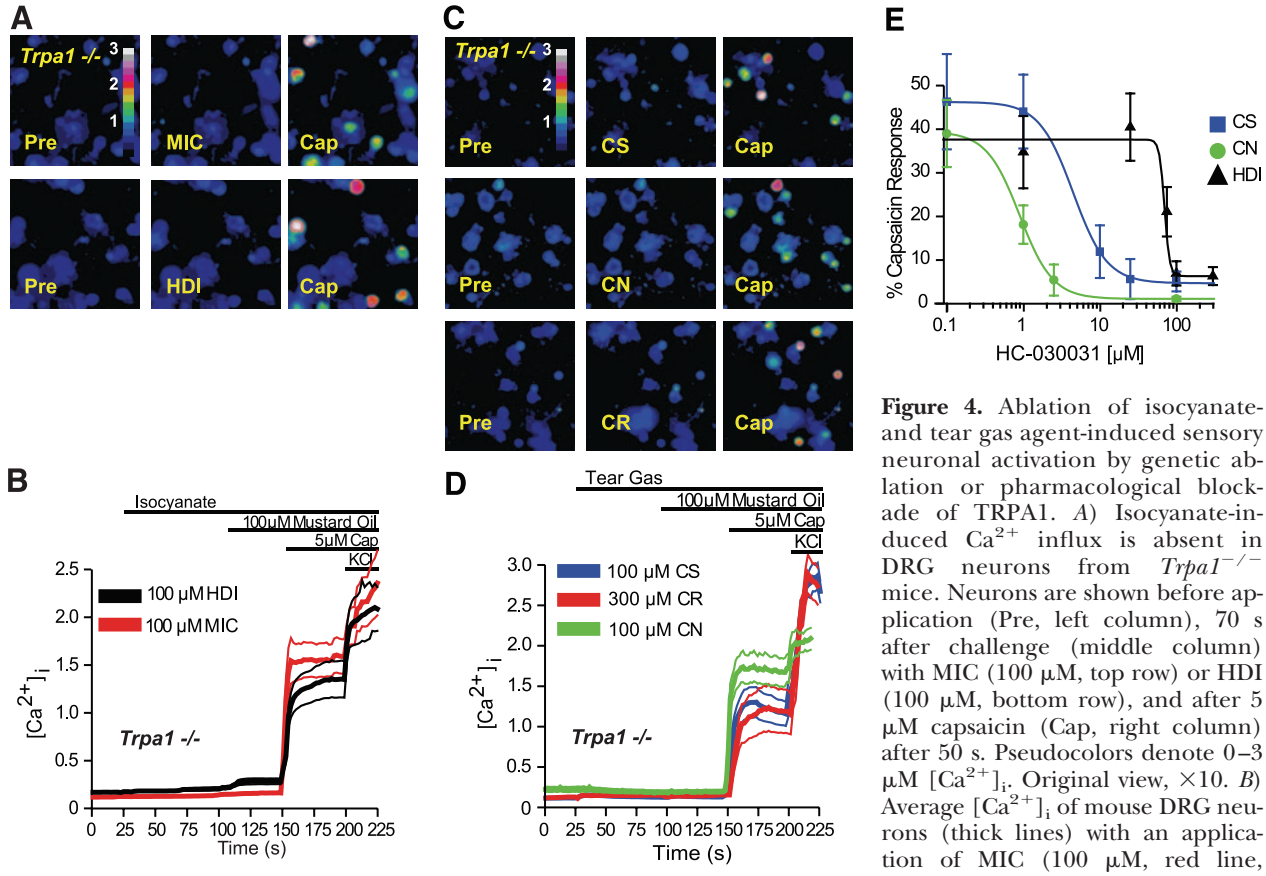

Figure 4. Ablation of isocyanate- and tear gas agent-induced sensory neuronal activation by genetic ablation or pharmacological blockade of TRPA1. *A*) Isocyanate-induced Ca^{2+} influx is absent in DRG neurons from *Trpa1*$^{-/-}$ mice. Neurons are shown before application (Pre, left column), 70 s after challenge (middle column) with MIC (100 μM, top row) or HDI (100 μM, bottom row), and after 5 μM capsaicin (Cap, right column) after 50 s. Pseudocolors denote 0–3 μM $[Ca^{2+}]_i$. Original view, ×10. *B*) Average $[Ca^{2+}]_i$ of mouse DRG neurons (thick lines) with an application of MIC (100 μM, red line, n=217 from 2 mice) and HDI (100 μM, black line, n=204 neurons from 2 mice), followed by 100 μM mustard oil, 5 μM capsaicin (Cap), and 65 mM KCl. Thin lines represent SE. *C*) Tear gas agent-induced Ca^{2+} influx is absent in DRG neurons from *Trpa1*$^{-/-}$ mice, shown before activation (Pre, left column), 70 s after challenge (middle column) with CS (100 μM, top row), CN (100 μM, middle row), or CR (300 μM, bottom row), followed by 5 μM capsaicin (Cap, right column) after 50 s. Pseudocolors denote 0–3 μM $[Ca^{2+}]_i$. Original view, ×10. *D*) Average $[Ca^{2+}]_i$ of mouse DRG neurons (thick lines) with an application of CS (10 μM, blue line, n=229 neurons from 2 mice), CN (100 μM, green line, n=270 neurons from 5 mice), or CR (300 μM, red line, n=108 neurons), followed by 100 μM mustard oil, 5 μM capsaicin (Cap), and 65 mM KCl. Thin lines represent SE. *E*) Dose-response curves of inhibition of industrial isocyanate or tear gas agent-activated Ca^{2+}-influx into mouse DRG neurons by the TRPA1 antagonist HC-030031. $[Ca^{2+}]_i$ induced by each dose is represented as percentage of $[Ca^{2+}]_i$ elicited by a saturating dose of capsaicin (5 μM, Cap) applied 125 s later (baseline $[Ca^{2+}]_i$ was subtracted). HC-030031 inhibited the $[Ca^{2+}]_i$ induced by 10 μM HDI (IC_{50}=74±3 μM, n=31±4 Cap-sensitive neurons/dose, black triangles), 10 μM CS (IC_{50}=4.5±0.4 μM, n=26±6/ dose, blue squares), and 10 μM CN (IC_{50}=884±23 nM, n=25±5/dose, green circles) in mouse DRG neurons.

therefore examined the effects of pharmacological inhibition and genetic ablation of TRPA1 on the behavioral responses to isocyanates and tear gas agents in mice. HDI, CN, and CS (100 mM each) caused immediate nocifensive responses on application to the mouse eye (MIC was too volatile and dangerous to test). The mice initially wiped their eyes and facial area and then continued with characteristic nocifensive behavior by vigorously stroking their heads and facial area against the bottom of the observation chamber (33). This behavior was completely absent when just vehicle was applied. We then injected the mice with the TRPA1 antagonist HC-030031 (300 or 50 mg/kg body weight i.p.) and applied the same dose of noxious chemical to the opposite eye 1 h later (300 mg/kg HC-030031 (n=6/group for CS and HDI; n=9 for CN) and 50 mg/kg HC-030031 (n=6/group). Remarkably, HC-030031 dramatically reduced the frequency of nocifensive responses to all three agents (**Fig. 5*A***). We then used a more conventional method of examining TRPA1-associated nocifensive responses, comparing nocifensive responses after intraplantar injections of HDI (6 mM) or the tear gas agent CN (4 mM) into the mouse hindpaw before and after treatment with 100 mg/kg body weight HC-030031. After the initial intraplantar injections, mice responded with immediate nocifensive behavior, including flinching, lifting, and licking of the paw (Fig. 5*B*). This behavior was greatly reduced in the same mice approximately 1 h after treatment with HC-030031 (Fig. 5*B*).

Because HC-030031 may inhibit the effects of isocyanates and tear gases in a nonspecific manner, we also compared isocyanate- and tear gas agent-induced behavior between TRPA1-deficient mice after eye application. Strikingly, nocifensive responses to tear gas agents (CN and CS) were completely absent in *Trpa1*$^{-/-}$ mice in this test (Fig. 5*C*). These results suggest that *Trpa1*$^{-/-}$ mice failed to detect tear gas agents as noxious stimuli. Responses to the isocyanate HDI were significantly abated (Fig. 5*C*). In addition to facial exposures, we compared responses of *Trpa1*$^{-/-}$ and *Trpa1*$^{+/+}$ mice after injections of the relatively soluble tear gas agents CN (n=8/group) and bromoacetone (n=6/group) into the hindpaw. After injections, wild-type mice responded with immediate nocifensive behavior, which was greatly reduced in *Trpa1*$^{-/-}$ mice (Fig. 5*D*).

In summary, our behavioral tests support an essential role for TRPA1 in the sensory detection of industrial isocyanates and tear gas agents (CN, CS, and bromoacetone) *in vivo*. Furthermore, exposure-related pain and irritation by these agonists can be prevented by TRPA1 antagonists.

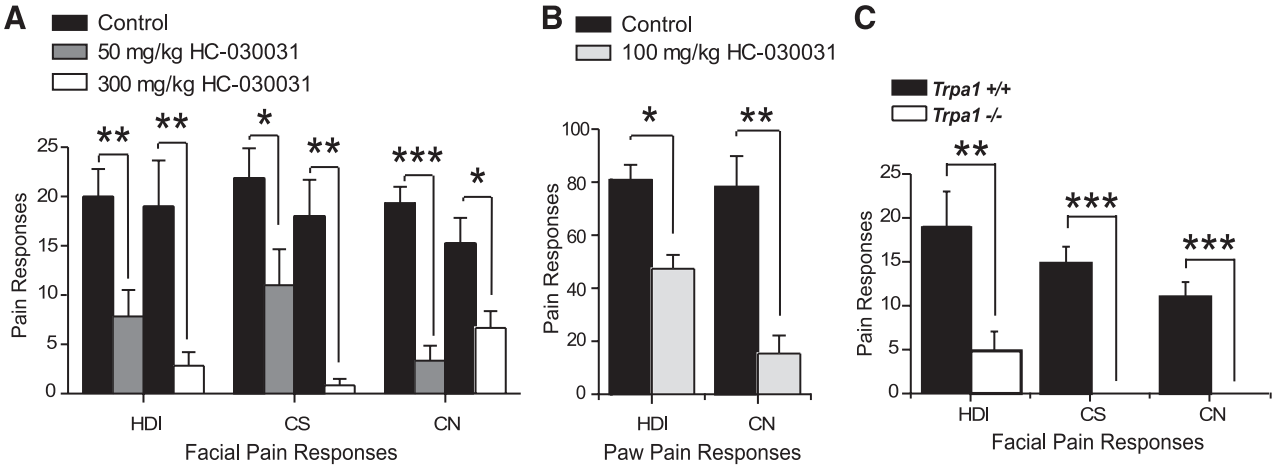

Figure 5. Ablation of isocyanate- and tear gas agent-induced nocifensive responses in mice by genetic deletion or pharmacological blockade of TRPA1. *A*) Nocifensive responses after application of 10 μl of 100 mM HDI (n=6), 100 mM CS (n=6), or 100 mM CN (n=9) to the eye of untreated C57/BL6 wild-type mice (black bars) and the same mice after an injection of 1 mg i.p.(50 mg/kg, gray bar) or 6 mg i.p.(300 mg/kg, white bar) of TRPA1 antagonist HC-030031. Nocifensive responses were quantified by counting strokes of the orbitofacial area on the observation chamber floor over 2 min for CS and CN for 300 mg/kg experiments and over 3 min for other treatments. Significance: $^{**}P < 0.01$; $^{*}P < 0.05$. *B*) Nocifensive responses (licks, lifts, and flicks) over 3 min after 25-μl subplantar injections of 4 mM CN or 6 mM HDI (n=6/group) into hindpaws of untreated C57/BL6 wild-type mice (black bars) and the same mice after an injection of 2 mg i.p.(100 mg/kg, gray bars). Significance: $^{**}P < 0.01$; $^{*}P < 0.05$. *C*) Comparison of nocifensive responses of wild-type (black bars) and *Trpa1*$^{-/-}$ mice (white bars) after application of 10 μl of 200 mM HDI, 100 mM CS, or 100 mM CN to right eye. Strokes of the orbitofacial area against the observation chamber floor were counted over 2 min for CS and CN and for 3 min for HDI (n=6 wild-type and n=6 *Trpa1*$^{-/-}$ mice were tested with CN, n=6 wild-type and n=7 *Trpa1*$^{-/-}$ mice tested with CS, and n=6 wild-type and n=7 *Trpa1*$^{-/-}$ mice were tested with HDI). $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. *D*) Nocifensive responses (licks, lifts, and flicks) over 5 min after 25-μl subplantar injections of 2 mM CN (n=8/group) or 4 mM bromoacetone (Br acetone, n=6/group) into hindpaws of *Trpa1*$^{+/+}$ and *Trpa1*$^{-/-}$ mice. $^{*}P < 0.05$.

DISCUSSION

Even though the release of methyl isocyanate in Bhopal caused the largest chemical industrial accident in history, the molecular basis of injury and irritation elicited by isocyanate exposures has remained unknown. In our present study, we have demonstrated that industrial isocyanates target the same sensory neuronal receptor as tear gas agents, TRPA1, to rapidly activate pain and sensory irritation. We find that TRPA1 channels expressed in primary sensory neurons and heterologous cells are robustly activated by both classes of agents. Isocyanate- and tear gas-induced nocifensive behavior is greatly reduced in TRPA1-deficient mice. Moreover, treatment of mice with newly developed TRPA1 antagonists leads to a dramatic reduction in sensitivity to isocyanates and tear gas agents.

Activation of TRPA1 by industrial isocyanates may have contributed to the acute and chronic health effects experienced by victims of the Bhopal incident and by agricultural and industrial laborers (1, 6). We show that industrial isocyanates strongly activate human TRPA1 channels and, in mice, have effects very similar to those of tear gases, activating trigeminal nerve endings in the eyes and facial area to elicit nocifensive responses. Trigeminal nerve fibers innervating the facial skin, mucous membranes, and eyes are the first line of defense against chemical exposures threatening tissue integrity and function (22). By acting similarly to tear gas agents, isocyanates induce ocular pain, lacrimation, and blepharospasm through trigeminal-autonomic and trigeminal-motor reflexes in exposed individuals. In addition to ocular and facial cutaneous nerve endings, isocyanates may also target TRPA1 channels in nerve endings lining the airways. In humans, activation of airway nerve endings by chemical irritants triggers cough, sneezing, airway mucus secretion, edema, and obstruction through activation of sensory nerves. In mice, these effects result in respiratory depression, significantly lowering respiratory rates (61). Recently, we showed that TRPA1 is essential for the activation of murine sensory neurons by the irritant chlorine and for chlorine-induced respiratory depression (36). Similar to chlorine, isocyanates and other TRPA1 agonists such as acrolein induce respiratory depression in rodents and other mammalian species, suggesting a crucial role of TRPA1 in this physiological response to chemical sensory irritation (14, 62, 63).

Our study is the first to provide a clear mechanistic basis for the biological actions of tear gases *in vivo*, supporting a central role of TRPA1 in the neuronal sensation of all major tear gas agents and subsequent activation of involuntary nocifensive reflex responses, including lacrimation, mucus secretion, and muscle contraction. We identify CS and CN as the most highly potent activators of heterologously expressed human TRPA1 channels. In our hands, CR agent is less potent than CS and CN, a finding that is in contrast to a recent study reporting a higher potency of CR on hTRPA1 *in vitro* (44). The reason for this discrepancy may lie in the differing purity of the agents used or in differences in experimental conditions.

We observed large differences in potencies of tear gas agents in heterologous cells and native sensory neurons. Although divergence of potencies have been observed for TRPA1 agonists before, we found that some tear gas agents have >100-fold higher potencies in human or mouse TRPA1-expressing HEK-293T cells than in mouse sensory neurons (36). In contrast, isocyanates show largely equal potencies in heterologous cells and native neurons. Our results indicate that *in vitro* studies alone are insufficient to evaluate specific TRPA1 agonist activity for a given chemical. We also found that previously identified covalent acceptor sites in TRPA1 are essential for activation by some agonists (CN and CR) but not by others (MIC, HDI, and CS). These results suggest that, in addition to electrophilic reactivity, other factors affect the ability of chemical agents to activate TRPA1. Some chemical agonists may bind to additional, as yet unidentified, covalent acceptor sites. Other agents may have different membrane permeabilities in heterologous cells or neurons, or their actions may be affected by intracellular reducing agents. Finally, responses by native TRPA1 channels may be affected by additional protein subunits, post-translational modifications, or differences in regulation of the local Ca^{2+} microenvironment (64).

The essential role of TRPA1 as the sole mediator of tear gas-related irritation *in vivo* is supported by our observation that TRPA1-deficient mice are largely impervious to the noxious effects of tear gases. In contrast to isocyanates, exposure to tear gas agents causes less tissue damage and long-term health effects. CS and CN are much less volatile than MIC and are usually dispersed as aerosols together with organic solvents or burned to reach irritating airborne concentrations (12). Nevertheless, adverse health effects and even deaths have been reported after tear gas exposures, especially when exposures occurred in closed environments. Responses include acute bronchospasm, pulmonary edema, asthma-like symptoms, and severe contact dermatitis (65–70). Individuals affected by preexisting allergic conditions seem to be especially prone to hypersensitivity reactions after tear gas exposures. In addition to the two major tear gas agents, TRPA1 is also activated by CR, benzyl bromide, bromoacetone, and PS. Presently, PS is widely used as a soil fumigant in agriculture, causing frequent occupational and environmental exposures (71, 72). TRPA1 activation is likely to contribute to the health effects caused by PS, including eye and respiratory tract irritation.

Irritant-induced sensory reflexes and pain are thought to be essential for the protection of eyes, skin, and airways from further chemical exposures. However, in the cases of isocyanates and tear gases, sensory responses usually occur rapidly and with very

high intensity, leading to partial or complete incapacitation. During the Bhopal incident, the TRPA1-mediated acute noxious effects of methyl isocyanate may thus have prevented many victims from escaping further exposure, leading to aggravated tissue damage due to the nonspecific corrosive effects of the toxicant. Individuals having airway infections or chronic inflammatory airway conditions, both highly prevalent in developing countries, may have responded more violently to MIC exposure. Activation of inflammatory signaling pathways in asthma, rhinitis, or airway infections could explain hypersensitivity responses to isocyanates and tear gases, because these pathways dramatically increase the sensitivity of TRPA1 to its agonists (9, 29, 30, 34, 56).

Individuals exposed to high levels of TRPA1 agonists, including chlorine and isocyanates, often present with RADS (73–75). RADS is characterized by highly increased sensitivity to chemical and physical stimuli, in addition to the initial sensitizing stimulus, resulting in asthma-like symptoms such as cough, wheezing, chest tightness and dyspnea (73). For example, agricultural workers exposed to MIC during a spill of the pesticide, metam sodium, subsequently became highly sensitive to diesel exhaust (5). Diesel exhaust contains high levels of the TRPA1 agonist, acrolein, and induced lacrimation, strong nasal irritation, and cough in the MIC-preexposed individuals (5). The multiple chemical sensitivity of TRPA1 readily explains the symptoms observed in patients with RADS. After an initial sensory challenge and tissue injury by a high-level chemical exposure, sensory TRPA1 channels become sensitized through inflammatory signaling pathways, establishing prolonged hypersensitivity to multiple reactive chemicals (29, 30, 34, 56). The role of TRPA1 in chemical hypersensitivity may extend to other, less clearly defined, conditions, including sensory hyperreactivity and multiple chemical sensitivity (76, 77).

RADS and related conditions are only partially responsive to the therapeutic interventions developed for the treatment of asthma. Our data suggest that TRPA1 antagonists may be effective in blocking the exaggerated chemosensory responses accompanying these conditions. Moreover, we show that TRPA1 antagonists prevent the acute sensory irritation elicited by exposures to isocyanates and tear gasses. TRPA1 antagonists may also be useful for postexposure treatment, reducing sensory irritation and, potentially, preventing adverse long-term health effects elicited by neurogenic inflammatory mechanisms. FJ

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A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma

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Asthma is an inflammatory disorder caused by airway exposures to allergens and chemical irritants. Studies focusing on immune, smooth muscle, and airway epithelial function revealed many aspects of the disease mechanism of asthma. However, the limited efficacies of immune-directed therapies suggest the involvement of additional mechanisms in asthmatic airway inflammation. TRPA1 is an irritant-sensing ion channel expressed in airway chemosensory nerves. TRPA1-activating stimuli such as cigarette smoke, chlorine, aldehydes, and scents are among the most prevalent triggers of asthma. Endogenous TRPA1 agonists, including reactive oxygen species and lipid peroxidation products, are potent drivers of allergen-induced airway inflammation in asthma. Here, we examined the role of TRPA1 in allergic asthma in the murine ovalbumin model. Strikingly, genetic ablation of TRPA1 inhibited allergen-induced leukocyte infiltration in the airways, reduced cytokine and mucus production, and almost completely abolished airway hyperreactivity to contractile stimuli. This phenotype is recapitulated by treatment of wild-type mice with HC-030031, a TRPA1 antagonist. HC-030031, when administered during airway allergen challenge, inhibited eosinophil infiltration and prevented the development of airway hyperreactivity. Trpa1$^{-/-}$ mice displayed deficiencies in chemically and allergen-induced neuropeptide release in the airways, providing a potential explanation for the impaired inflammatory response. Our data suggest that TRPA1 is a key integrator of interactions between the immune and nervous systems in the airways, driving asthmatic airway inflammation following inhaled allergen challenge. TRPA1 may represent a promising pharmacological target for the treatment of asthma and other allergic inflammatory conditions.

airway hyperreactivity | TRP channel | TRPA1

The dramatic increase in the number of asthma cases over the last decades is of great concern for public health in the United States and world-wide (1, 2). The inflammatory response in asthma is orchestrated by CD4 T_h2 cells inducing eosinophil infiltration and mast cell activation, followed by tissue remodeling, mucus hypersecretion, and airway hyperresponsiveness (3). While it is clear that immune mechanisms play a significant role in the development and maintenance of asthma, the limited efficacy of immune therapies suggests the involvement of additional mechanisms and physiological systems in the disease process (4).

The airways are densely innervated by peripheral sensory neurons expressing specific receptors activated by noxious chemicals contained in the inhaled air (5). Over the last decades, evidence has mounted for bi-directional feedback between immunogenic and neurogenic mechanisms in airway inflammation (6, 7). Neuronal activation causes pain and irritation, neurogenic inflammation, mucus secretion, and reflex responses, such as cough, sneezing, and bronchoconstriction (8, 9). Members of the transient receptor potential (TRP) superfamily of ion channels play a key role in the response of sensory neurons to inflammatory mediators (10–12). The 2 major pro-inflammatory TRP ion channels in sensory neurons are TRPV1, the capsaicin receptor, and TRPA1, activated by mustard oil (13–16).

Agonists of TRPV1 and TRPA1, such as capsaicin, acrolein, or chlorine, are potent tussive agents and have been associated with allergic and occupational asthma and reactive airway dysfunction syndrome (RADS) (12, 17–23). Potential endogenous TRPA1 agonists include reactive oxygen species, hypochlorite, and lipid peroxidation products (18, 24–26). Similar to TRPV1, TRPA1 is activated or sensitized downstream of inflammatory PLC-coupled receptor pathways and mediates inflammatory pain sensitization (12–14, 27). In animal models, TRPA1 antagonists block chemically induced inflammatory thermal and mechanical hyperalgesia, neuropathic pain, and diminish acute airway responses to chemical exposures (17, 19, 28).

The roles of TRPV1 and TRPA1 in asthmatic airway inflammation remain unknown. Using a murine model of acute asthma, we identify a critical role of TRPA1 in this disease. We show that genetic deletion of TRPA1 or pharmacological channel inhibition diminishes allergen-induced inflammatory leukocyte infiltration, mucus production, cytokine and chemokine levels, and airway hyperreactivity. Trpa1$^{-/-}$ mice also show impaired acute and inflammatory neuropeptide release in the airways. In contrast, all aspects of asthmatic airway inflammation were normal in Trpv1$^{-/-}$ mice. These results suggest that TRPA1 is a major neuronal mediator of allergic airway inflammation and may represent a promising target for suppression of inflammation and airway hyperreactivity in asthma.

Results

Diminished Leukocyte Airway Infiltration and Airway Hyperreactivity in OVA-Challenged Trpa1$^{-/-}$ Mice. We used the ovalbumin (OVA) mouse model of asthma to induce a T_h2-directed allergic response, comparing leukocyte levels in the bronchoalveolar lavage fluid (BALF) of OVA-challenged wild-type, Trpa1$^{-/-}$, and Trpv1$^{-/-}$ mice (Fig. 1*A* and *B*). Leukocyte numbers were greatly elevated in BALF of OVA-challenged wild-type C57BL/6 mice

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Conflict of interest statement: S.-E.J. is serving on the scientific advisory board of Hydra Biosciences, Cambridge, MA. Hydra Biosciences developed the TRPA1-antagonist, HC-030031, used in the present study. D.d.C., M.D., J.S.W., C.M.F., J.A.C., N.J.H., and M.M.M. are employees of Hydra Biosciences, and receive options.

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PHARMACOLOGY

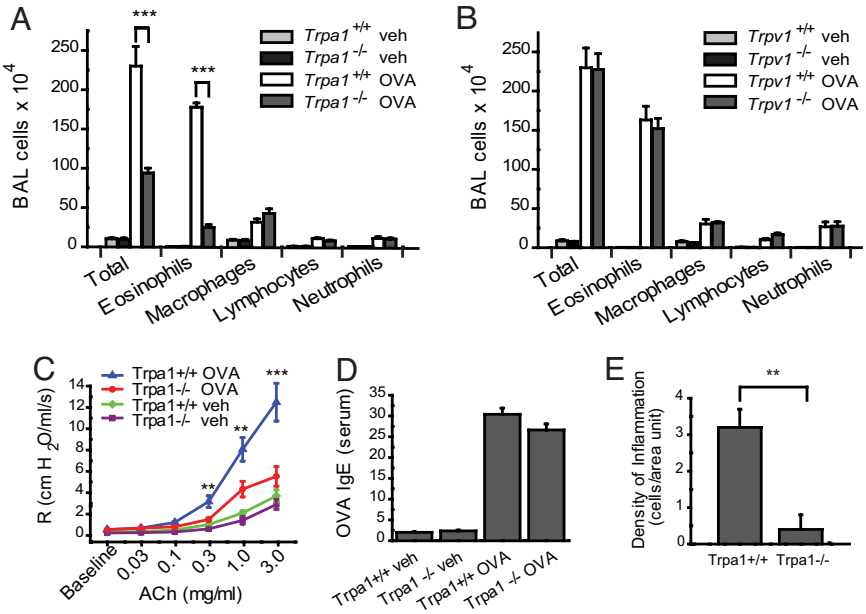

Fig. 1. Decreased inflammatory response to inhaled OVA in TRPA1-deficient mice. (*A*) Reduced leukocyte infiltration in airways of OVA-challenged Trpa1$^{-/-}$ mice. Cell differentials are shown for total cells, eosinophils, macrophages, lymphocytes, and neutrophils in BALF collected from vehicle (veh, PBS)- and OVA-challenged Trpa1$^{+/+}$ and Trpa1$^{-/-}$ mice. Animal groups: Trpa1$^{+/+}$ OVA: $n = 8$, Trpa1$^{-/-}$ OVA: $n = 8$, Trpa1$^{+/+}$ veh: $n = 7$, Trpa1$^{-/-}$ veh: $n = 6$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (*B*) Normal inflammatory leukocyte infiltration in OVA-challenged Trpv1$^{-/-}$ mice. BALF leukocyte cell differentials are shown for vehicle (veh, PBS)- and OVA-challenged Trpv1$^{+/+}$ and Trpv1$^{-/-}$ mice. Animal groups: Trpv1$^{+/+}$ OVA: $n = 6$, Trpv1$^{-/-}$ OVA: $n = 4$, Trpv1$^{+/+}$ veh: $n = 6$, Trpv1$^{-/-}$ veh: $n = 4$. (*C*) Comparison of airway resistance (*R*) in OVA-challenged Trpa1$^{+/+}$ (blue) and Trpa1$^{-/-}$ mice (red), as well as vehicle (veh)-treated Trpa1$^{+/+}$ (green) and Trpa1$^{-/-}$ (purple) mice, measured by forced oscillation in response to increasing dosages of acetylcholine. Animal groups: Trpa1$^{+/+}$ OVA: $n = 4$, Trpa1$^{-/-}$ OVA: $n = 4$, Trpa1$^{+/+}$ veh: $n = 6$, Trpa1$^{-/-}$ veh: $n = 6$. (*, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$). (*D*) Induction of OVA-reactive Ig E in OVA-challenged wild-type and TRPA1-deficient mice, as determined by ELISA. Animal groups as in Fig. 1 *A*. (*E*) Density of inflammation in H&E-stained airway sections from OVA-challenged Trpa1$^{+/+}$ and Trpa1$^{-/-}$ mice, scored by counting of inflammatory cells near bronchial bundles ($n = 4$ mice per group).

(Fig. 1*A*). Eosinophils represented the majority of leukocytes (Fig. 1*A*). In OVA-challenged TRPA1-deficient mice, we observed a remarkable reduction in BALF leukocyte numbers (>50%), with eosinophilia reduced by >80% (Fig. 1*A*). In contrast, OVA-challenged Trpv1$^{-/-}$ mice showed robust leukocyte infiltration, with BALF cell counts indistinguishable from those of wild-type mice (Fig. 1*B*).

Airway hyperreactivity (AHR) is another important hallmark of asthma. Airway resistance was measured by forced oscillation in response to i.v. administration of increasing concentrations of acetylcholine (Fig. 1*C*). OVA-challenged wild-type C57BL/6 mice developed robust AHR (Fig. 1*C*). In OVA-challenged Trpa1$^{-/-}$ mice, AHR was very mild, only differing from control animals at the highest doses of acetylcholine. Basal reactivity of vehicle-treated wild-type and Trpa1$^{-/-}$ mice was identical. We conclude that TRPA1 plays an essential role in asthma-related airway hyperreactivity.

Airway eosinophilia and hyperreactivity are consequences of an allergen-induced T_h2-lymphocyte response leading to the production of allergen-specific IgE antibodies. We measured OVA-reactive IgE in serum by EIA to verify whether Trpa1$^{-/-}$ mice produce a normal T_h2-response following immunization and airway challenge with OVA (Fig. 1*D*). OVA-reactive IgE serum levels in Trpa1$^{-/-}$ mice were indistinguishable from those in wild-type C57BL/6 mice, suggesting a normal T_h2-dependent systemic immune response to OVA (Fig. 1*D*). These data indicate that TRPA1 has a crucial role in later events leading to airway inflammation following allergen challenge.

Quantitative comparison of inflammatory cell densities near airways in lung sections of OVA-challenged mice confirmed diminished eosinophilia in Trpa1$^{-/-}$ mice and reduced hyperplasia of mucus-producing goblet cells (Fig. 1*E* and *supporting information* (SI) Fig. S1*A*).

Reduced Mucus Production and T_h2 Cytokine Levels in Airways of OVA-Challenged Trpa1$^{-/-}$ Mice. Using quantitative Taqman PCR, we compared the transcriptional levels of the muc5ac mucin genes in whole lung cDNA (Fig. 2*A*). Mucins are mucus proteins highly expressed in asthmatic airways. OVA-challenged wild-type mice displayed robust induction of muc5ac transcription (Fig. 2*A*). In contrast, muc5ac levels were reduced by 50% in lungs of OVA-challenged Trpa1$^{-/-}$ mice (Fig. 2*A*). Mucin5ac induction was normal in OVA-challenged Trpv1$^{-/-}$ mice (Fig. S1*B*).

T_h2 leukocytes orchestrate the allergic inflammatory response in the airways through the release of cytokines, such as interleukin 5 (IL-5). We examined transcriptional activity of the IL-5 gene by Taqman PCR of whole lung cDNA from wild-type, Trpa1$^{-/-}$, and Trpv1$^{-/-}$ mice as a measure for T_h2 leukocyte infiltration and activity (Fig. 2*B*). Strikingly, while OVA-challenged wild-type mice showed a robust increase in IL-5 transcriptional activity, IL-5 levels in OVA-challenged Trpa1$^{-/-}$ mice were indistinguishable from those in vehicle-treated mice (Fig. 2*B*). Trpv1$^{-/-}$ mice showed normal induction of IL-5 expression (Fig. S1*C*).

A systematic comparison of peptide concentrations of cytokines and chemokines was performed using Luminex multiplex protein analysis of BAL fluid (Fig. 2*C*). As predicted from our qPCR analysis, IL-5 protein levels in BALF of OVA-challenged Trpa1$^{-/-}$ mice were much lower (<20%) than in wild-type mice (Fig. 2*C*, *Inset*). Trpa1$^{-/-}$ mice also showed significantly diminished levels of IL-13, IL-17, eotaxin, MCP-1, RANTES, and TNFα, suggesting a profound defect in the T_h2-directed local inflammatory response in the airways (Fig. 2*C*). Levels of IFN γ, an indicator for a T_h1 leukocyte activity, were below the detection limit in all mouse groups, showing that the observed reduction in airway eosinophilia was not due to a shift toward a T_h1-directed immune response in Trpa1$^{-/-}$ mice. Cytokine

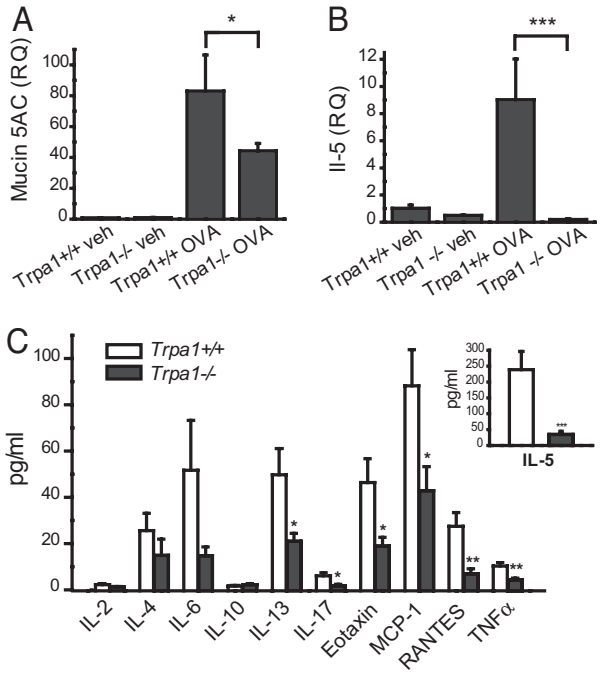

Fig. 2. Impaired induction of mucin, cytokines, and chemokines in OVA-challenged airways of Trpa1-deficient mice. (*A*) Relative quantities (RQ) of mucin5ac gene transcript, determined by Taqman qPCR of whole mouse lung cDNA. Mucin5ac induction is diminished in Trpa1$^{-/-}$ OVA mice. GAPDH transcript levels were used for normalization as endogenous control. Animal groups: Trpa1$^{+/+}$ veh: $n = 4$, Trpa1$^{-/-}$ veh: $n = 4$, Trpa1$^{+/+}$ OVA: $n = 6$, Trpa1$^{-/-}$ OVA: $n = 7$. *, $P < 0.05$. (*B*) Relative quantities (RQ) of interleukin 5 (IL-5) gene transcript, as determined by Taqman real-time quantitative PCR of whole mouse lung cDNA. OVA-challenged Trpa1$^{-/-}$ mice show no significant changes in IL-5 transcription compared with vehicle-treated mice. GAPDH transcript levels were used for normalization as endogenous control. Animal groups: Trpa1$^{+/+}$ veh: $n = 4$, Trpa1$^{-/-}$ veh: $n = 4$, Trpa1$^{+/+}$ OVA: $n = 6$, Trpa1$^{-/-}$ OVA: $n = 6$. ***, $P < 0.001$. (*C*) Comparison of cytokine and chemokine levels in BALF of OVA-challenged Trpa1$^{+/+}$ (white) and Trpa1$^{-/-}$ (black) mice, as measured by Luminex peptide analysis. Groups: Trpa1$^{+/+}$ $n = 8$–10, Trpa1$^{-/-}$ $n = 8$–10 for each analyte. **, $P < 0.01$; ***, $P < 0.001$

levels were normally elevated in OVA-challenged Trpv1$^{-/-}$ mice (Fig. S1*D*).

A TRPA1 Antagonist Reduces Airway Inflammation and Hyperreactivity when Administered During OVA Airway Challenge. TRPA1-antagonists showed efficacy in animal models of acute and inflammatory pain and diminished the noxious effects of TRPA1 agonists known to cause asthma-related conditions (17, 19, 20, 28, 29). We asked whether a TRPA1 antagonist would prevent or diminish airway inflammation when administered to OVA-sensitized Balb/C mice during the airway challenge phase of the OVA protocol. HC-030031, the most thoroughly studied TRPA1 antagonist, was injected i.p. into OVA-sensitized animals on the day before (200 mg/kg) and twice daily (100 mg/kg) during the 4 days of OVA airway challenge. While vehicle (methyl cellulose, MC)-treated mice showed robust OVA-induced increases in BALF leukocyte numbers, cell numbers were diminished in OVA-challenged HC-030031-treated mice (Fig. 3*A*). Moreover, treatment with HC-030031 led to a nearly complete suppression of airway hyperreactivity in OVA-challenged Balb/C mice (Fig. 3*B*). Mucin5ac transcription was strongly suppressed by HC-030031 treatment, indicating diminished production of airway mucus (Fig. 3*C*). Antagonist-injected mice also showed diminished levels of T$_h$2 cytokines IL-5 and IL-13 in BALF (Fig. 3*D*). Histological sections of airways from antagonist-treated mice showed much lower densities of inflammatory cells (Figs. 3*E* and S1*E*). Similar to Trpa1$^{-/-}$ mice, treatment with HC-030031 did not affect serum levels of OVA-specific IgE in OVA-challenged wild-type BALB/C mice, indicating a normal T$_h$2-directed systemic inflammatory response (Fig. S1*F*). These data suggest that TRPA1 plays a crucial role in the development of asthma during airway allergen challenge, enabling inflammatory leukocyte infiltration, airway hyperreactivity, and mucus production.

Trpa1 is Essential for Chemically Induced and Inflammatory Neuropeptide Release in the Airways. It is unclear whether the pro-inflammatory action of TRPA1 in asthma can be explained through purely neurogenic effects. TRPA1 may play an as yet undetected role in cells of the immune system or in airway tissue. To assess this possibility, we used Taqman quantitative PCR to compare TRPA1 transcript levels in cDNA derived from spleen harboring a large variety of leukocyte precursors, T$_h$2 lymphocytes, whole mouse lung and BALF leukocytes of OVA-challenged mice, and DRG. Relative transcript quantities in spleen, T$_h$2 cells, whole lung, and leukocytes were minimal, with DRG expression several 100-fold higher (Fig. S1*G*). Additional qPCR experiments using cDNA prepared from primary leukocytes and leukocyte cell lines failed to detect the presence of TRPA1 cDNA. These results point to a key role for sensory neuronal TRPA1 channels in allergic airway inflammation.

TRPA1 may be a critical trigger for neuropeptide release crucial for leukocyte infiltration and inflammatory progression in asthmatic airways. To investigate this possibility, we compared neuropeptide release in airways of wild-type and and Trpa1 $^{-/-}$ mice in response to 2-chloroacetophenone (CN), a potent inflammatory TRPA1 agonist (20). We performed a 30-s BAL in mice with CN (4 mM) contained in the BAL buffer (PBS) and measured the resultant release of CGRP, substance P (SP) and neurokinin A (NKA) using EIA (Fig. 4 *A*–*C*). CN induced strong increases in the levels of all 3 neuropeptides in BALF of wild-type C57BL/6 mice (Fig. 4 *A*–*C*). In Trpa1$^{-/-}$ mice CN-induced peptide release was clearly diminished (<50% of wild-type levels), supporting a specific and essential role for TRPA1 in chemically stimulated neurogenic peptide release in the airways (Fig. 4 *A*–*C*). Acute CN-induced neuropeptide release was suppressed by prior treatment with HC-030031 in Balb/C mice (Fig. 4*D*).

Exogenous TRPA1 agonists such as CN are likely to mimic the actions of endogenous reactive products and inflammatory signaling pathways activating TRPA1 (16). Since Trpa1$^{-/-}$ mice showed clear deficiencies in acute neurogenic peptide release in the airways, we asked whether neuropeptide levels would also be reduced during airway allergen challenge. We compared neuropeptide levels in BALF of OVA-challenged wild-type and Trpa1$^{-/-}$-deficient C57/BL6 mice, and in antagonist treated Balb/C mice, focusing on NK-A, the most abundant neuropeptide in airway lining fluid (30) (Fig. 4 *E* and *F*). Indeed, we observed that the OVA-induced increase in BALF NK-A levels was clearly diminished in Trpa1$^{-/-}$ mice and in antagonist-treated Balb/C mice (Fig. 4 *E* and *F*).

Discussion

Our results reveal a crucial role for the sensory neuronal ion channel TRPA1 in experimental asthma. TRPA1-deficient mice showed profound deficits in airway infiltration by inflammatory leukocytes in the OVA model of allergic airway inflammation, accompanied by a reduction in inflammatory T$_h$2 cytokines, such as IL-5 and IL-13, and pro-inflammatory chemokines, such as TNFα and the eosinophil attractant, eotaxin. As a consequence, OVA-induced mucus production is impaired in Trpa1$^{-/-}$ mice. Airway hyperreactivity, another important hallmark of asthma, was strongly reduced. Pharmacological inhibition of TRPA1 during the airway challenge phase of the OVA

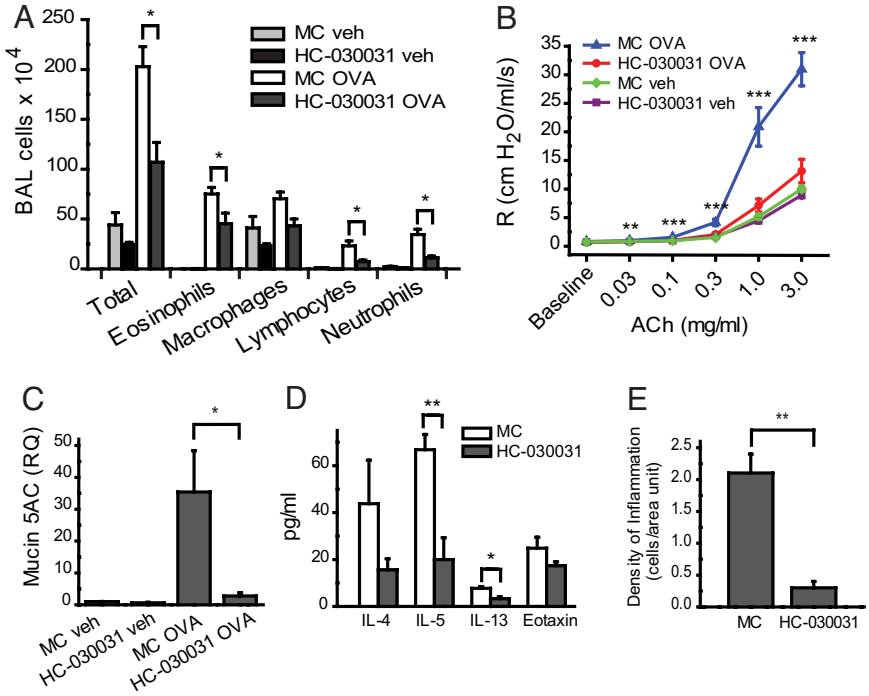

Fig. 3. Decreased inflammatory response in Balb/C mice treated with the TRPA1 antagonist HC-030031 during the OVA airway challenge phase. (*A*) Cell differentials for total leukocytes, eosinophils, macrophages, lymphocytes, and neutrophils in BALF collected from vehicle (veh, PBS)- or OVA-challenged mice injected i.p. with HC-030031 or with methyl cellulose (MC) during the airway challenge phase. Animal groups: MC veh: $n = 8$, HC-030031 veh: $n = 9$, MC OVA, $n = 10$, HC-030031 OVA: $n = 8$. *, $P < 0.05$. (*B*) Comparison of airway hyperresponsiveness to i.v. acetylcholine in vehicle (veh, PBS) - or OVA-challenged mice injected i.p. with HC-030031 or with just methyl cellulose (MC) during OVA airway challenge. Animal groups: MC veh: $n = 7$, HC-030031 veh: $n = 7$, MC OVA, $n = 7$, HC-030031 OVA: $n = 6$ (*, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$). (*C*) Decreased lung mucin5ac transcription in OVA-challenged mice treated with TRPA1 antagonist HC-030031, determined by Taqman q PCR. RQ of mucin5ac transcript are shown for vehicle (veh.) or OVA-challenged mice injected i.p. with HC-030031 or with just methyl cellulose (MC) during OVA airway challenge. GAPDH transcript levels were used for normalization as endogenous control. Animal groups: MC veh: $n = 4$, HC-030031 veh: $n = 4$, MC OVA, $n = 8$, HC-030031 OVA: $n = 8$. *, $P < 0.05$. (*D*) Cytokine and eotaxin levels in bronchoalveolar lavage fluid (BALF) of OVA-challenged Balb/C mice treated with TRPA1 antagonist HC-030031 (black) or carrier methyl cellulose (white). ($n = 4$ mice/group) *, $P < 0.05$; **, $P < 0.01$. (*E*) Density of inflammation in H&E-stained airway sections from OVA-challenged HC-030031-treated and −untreated (MC) Balb/C mice, scored by counting of inflammatory cells near bronchial bundles ($n = 4$ mice per group).

protocol confirmed the essential function of this receptor, blocking leukocyte infiltration, cytokine and neuropeptide release, mucus production, and abolishing airway hyperreactivity.

Trpa1$^{-/-}$ mice are deficient in the neuronal detection of multiple pro-inflammatory exogenous and endogenous agents. These include asthma-inducing agents such as chlorine, unsaturated aldehydes in smoke and smog, chloramines, tear gas agents, and industrial isocyanates, as well as endogenous reactive oxidative species and lipid mediators (12, 17–20, 24, 25). Some of these endogenous mediators are produced by infiltrating leukocytes or inflamed airway tissue and can reach concentrations high enough to chronically activate TRPA1 in airway nerve endings (31). Chemosensory deficiency in Trpa1$^{-/-}$ mice may cause a lack of neuronal excitation and Ca^{2+} influx activated by these reactive compounds during inflammatory progression in asthma, resulting in reduced reflex hyperreactivity and neuropeptide release. Indeed, we find that sensory neuropeptide release, a prerequisite for inflammatory leukocyte infiltration in mice, is impaired in the airways of Trpa1$^{-/-}$ mice. This defect applies to both acute neuropeptide release, induced by a TRPA1 agonist, and inflammatory peptide release following OVA challenge in the airways.

The ability of a TRPA1 antagonist to recapitulate the knockout phenotype suggests that TRPA1 fulfills an acute role in promoting local inflammation in the airways, rather than causing a developmental defect in immune system function. Administration of HC-030031 during the OVA airway challenge phase was sufficient to potently suppress airway leukocyte infiltration, mucus production and hyperreactivity. The role of TRPA1 in local inflammatory responses to allergen challenge is also supported by the observation that genetic deletion of TRPA1, or treatment with the TRPA1 antagonist HC-030031, did not seem to affect the systemic T_h2-mediated response to allergen immunization, as evidenced by normal serum levels of OVA-reactive IgE. Future experiments are needed to address the detailed mechanistic role of TRPA1 in neurogenic responses affecting T_h2 lymphocyte migration into the airways, cytokine production, leukocyte recruitment, as well as in airway hyperreactivity.

Our experiments clearly show that TRPV1, the capsaicin receptor, is not required for allergic airway inflammation in the OVA mouse model of asthma. A TRPA1-specific stimulus, possibly a reactive TRPA1-specific mediator, appears to be required to drive the pro-inflammatory activity of airway C-fibers in asthma. Nevertheless, TRPV1 is clearly involved in the symptomatic consequences of airway inflammation, as evidenced by a recent report showing anti-tussive activity of a TRPV1 antagonist in a model of chronic cough (32).

The data in our present study support the idea that TRPA1 may function as an integrator of chemical and immunological stimuli modulating inflammation in the airways. This integrative activity can explain the pro-inflammatory effects of chemical exposures in asthma patients (16). By activating TRPA1, chemical irritants may trigger the release of neuropeptides and chemokines in the airways, thereby exacerbating the cellular and tissue inflammatory response observed in our present study. Our study opens an avenue for asthma pharmacology, revealing TRPA1 as a potential target for anti-asthmatic drugs. Future studies will

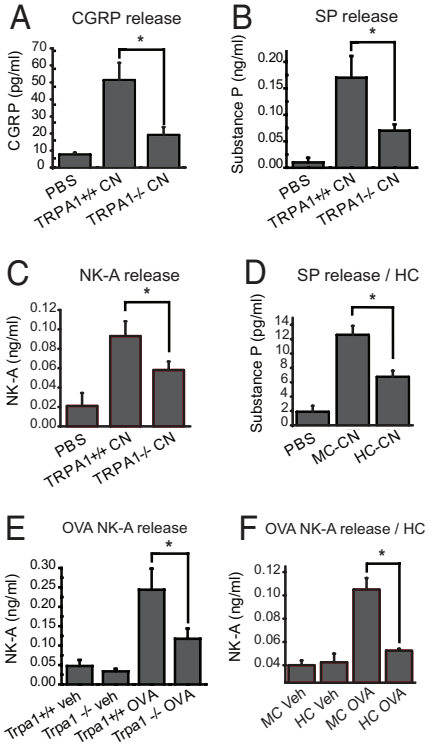

Fig. 4. Role of TRPA1 in chemically induced and inflammatory neuropeptide release in the airways, measured by EIA. (*A*) Diminished chemically induced release of CGRP in Trpa1$^{-/-}$ mice following lung exposure to CN (2-chloroacetophenone) during BAL. Averaged CGRP levels in BAL fluid of TRPA1$^{+/+}$ mice, treated with PBS ($n = 4$) or 4 mM CN ($n = 6$), and TRPA1$^{-/-}$ mice treated with 4 mM CN ($n = 5$) are shown. *, $P < 0.05$. (*B*) Diminished chemically induced release of Substance P (SP) in Trpa1$^{-/-}$ mice following lung exposure to CN (2-chloroacetophenone) during BAL. Treatments and mouse groups as in Fig. 4*A*. (*C*) Diminished chemically induced release of neurokinin A (NK-A) in Trpa1$^{-/-}$ mice following lung exposure to CN (2-chloroacetophenone) during BAL. Treatments and mouse groups as in Fig. 4*A*. (*D*) Diminished CN-induced release of SP in mice injected i.p. with TRPA1 antagonist HC-030031. Averaged SP concentrations are shown in BAL fluid of Balb/C mice treated with PBS ($n = 4$), 200 μM CN and methylcellulose vehicle (MC CN, $n = 4$), or with 200 μM CN and HC-030031 (HC CN, $n = 4$) are shown. *, $P < 0.05$. (*E*) Reduced level of neurokinin A in BAL fluid of OVA-challenged Trpa1$^{-/-}$ mice. NK-A levels were compared by EIA in BAL fluid of vehicle-treated wild-type mice (Trpa1$^{+/+}$ veh, $n = 11$), vehicle-treated Trpa1$^{-/-}$ mice ($n = 7$), OVA-challenged wild-type mice (Trpa1$^{+/+}$ OVA, $n = 12$), and OVA-challenged Trpa1$^{-/-}$ mice ($n = 12$). *, $\alpha < 0.05$. (*F*) Reduction of NK-A in BAL fluid of OVA-challenged Balb/C mice due to injection of TRPA1-antagonist HC-030031. NK-A levels were compared by EIA in BAL fluid of methyl cellulose and vehicle-treated mice (MC veh, $n = 4$), HC-030031- and vehicle-treated (HC veh, $n = 4$), methyl cellulose treated and OVA-challenged (MC OVA, $n = 7$), and HC-030031-treated and OVA-challenged mice (HC OVA, $n = 8$). *, $\alpha < 0.05$

address the action of TRPA1 antagonists in additional animal models of asthma and in other allergic inflammatory conditions.

Materials and Methods

Animals. Experimental procedures were approved by the Institutional Animal Care and Use Committees of Yale University, the University of California, San Francisco, and Hydra Biosciences. Mice were housed at facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in standard environmental conditions (12-h light-dark cycle and 23 °C). Food and water were provided ad libitum. Trpa1$^{-/-}$ mice were a gift from David Julius (University of California, San Francisco). The Trpa1-knockout allele was backcrossed into the C57BL/6 background (>99.5%) by marker assisted accelerated backcrossing (Charles River Laboratories). Trpv1$^{-/-}$ mice were purchased from Jackson Laboratories and C57/Bl6 and BALB/c mice from Charles River Laboratories. For experiments on C57BL/6, Trpv1$^{-/-}$, and Trpa1$^{-/-}$ mice, animals were matched for age (12–22 weeks) and gender. Six- to 8-week-old BALB/c mice were used for OVA sensitization and antagonist studies.

Sensitization and Airway Challenge Procedure. Mice were sensitized on days 0, 7, and 14 by i.p. injection of 50 μg ovalbumin (OVA) (Sigma-Aldrich) adsorbed in 2 mg alum gel (Sigma-Aldrich) in a total volume of 200 μL PBS. Control animals received alum only. Subsequently, lightly anesthetized mice (isoflurane) were intranasally challenged with OVA [100 μg in 40 μL PBS (PBS)] or with PBS alone on days 21, 22, and 23. For therapeutic intervention with TRP channel antagonist HC-030031, mice were given the compound (200 mg/kg mouse body weight) i.p. once on day 20 and 100 mg/kg twice a day on days 21, 22, and 23. On the day of lung mechanics measurement, all mice received 100 mg/kg compound once in the morning and subject to the end point of the experiment. All measurements and sample collection were performed 24 h after the final intranasal challenge. Following lung mechanics measurements plasma levels of HC-030031 along with samples of dose solutions were analyzed by LC/MS/MS using a standard protein precipitation extraction with the addition of an internal standard.

Measurement of Airway Reactivity. Twenty-four hours following the last OVA challenge, mice were anesthetized with pentobarbital (60 mg/kg of body weight) and urethane (1 g/kg). A tracheostomy was performed, and the trachea was cannulated. Mice were attached to a Flexivent pulmonary mechanics analyzer (SCIREQ) and ventilated at a tidal volume of 9 mL/kg, at a frequency of 150 bpm. Positive end-expiratory pressure was set at 2 cm H_2O. Mice were paralyzed with pancuronium (0.1 mg/kg i.p.). A 27-gauge needle was used to administer acetylcholine (0.03, 0.1, 0.3, 1.0, and 3.0 mg/mL) through the subclavial/tail vein to generate a concentration-response curve. Measurements of airway mechanics were made continuously applying the single-compartment model.

Quantitative Analysis of Cytokines, Chemokines, and Neuropetides in BAL Fluid. Cytokines and chemokines in BAL fluid (50 μL) were measured using a Milliplex MAP Mouse Cytokine/Chemokine Kit (Millipore) on Luminex 200 analyzer (Luminex), following the manufacturer's recommendations. Neuropeptide levels in BAL were measured by EIA (CGRP: Cayman Chemical; Substance P: Phoenix Pharmaceuticals; Neurokinin A: Bachem). To measure acute peptide release, 4 mM CN (2-chloroacetophenone) was added to the BAL buffer. Lungs were inflated with CN-BAL buffer for 30 sec. HC-030031 was injected i.p. in Balb/C mice at 200 mg/kg 24 h, 100 mg/kg 6 h, and 100 mg/kg 30 min before CN challenge (200 μM).

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Identification of Compounds Formed During Low Temperature Thermal Dispersion of Encapsulated o-Chlorobenzylidene Malononitrile (CS Riot Control Agent)

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U.S. Army chemical mask confidence training is conducted in an enclosed chamber where airborne o-chlorobenzylidene malononitrile (also known as CS or "tear gas") is generated using a low temperature (150–300°C) dispersal method. CS capsules are placed onto a flame-heated aerosol generator that melts the capsules and disperses CS into the chamber. To instill confidence in chemical protective equipment, trainees are required to break the seal of their chemical protective mask, resulting in the immediate irritation of their eyes, nose, throat, and lungs. Solid phase micro extraction (SPME) sample collection techniques were used inside the chamber, followed by gas chromatography and mass spectrometry (GC/MS) to identify unintended thermal degradation products created during the CS dispersal process. The temperature of the aerosol generator averaged 257°C, and 17 thermal degradation products were identified. To characterize the relationship between temperature and the types of CS thermal degradation products formed, CS was dispersed in a tube furnace at controlled temperatures from 150–300°C and analyzed using the same method. There was a graded response between temperature and the number of thermal degradation products formed, with one product formed at 150°C and 15 products formed at 300°C. Two additional products were identified in the chamber experiment when compared with the tube furnace experiment. These products are likely the result of molten CS dripping directly into the aerosol generator's flame, which averaged 652°C. To prevent undesirable degradation products during thermal dispersion of CS, a delivery system designed to contain the molten CS and maintain a consistent temperature near 150°C is recommended.

Keywords chlorobenzylidene, confidence chamber, CS, malononitrile, riot agent, thermal degradation

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INTRODUCTION

Riot control agents (RCAs), often referred to as "tear gas," are compounds designed to cause temporary incapacitation by causing strong irritation to the eyes, mouth, throat, lungs, and skin.[1,2] The U.S. military and most law enforcement agencies worldwide use o-chlorobenzylidene malononitrile (CS) as their standard RCA.[1–3] Individuals entering the U.S. Army are exposed to CS during initial chemical, biological, radiological, and nuclear (CBRN) mask confidence training and periodically during refresher training.[4] During this training, CS is thermally released into a mask confidence chamber by CS capsules being placed onto a metal surface (typically, an inverted coffee can) while the metal is heated with an open flame heat source.[5]

Soldiers equipped with chemical protective masks enter the chamber and perform various exercises intended to give them confidence in the protective mask's ability to shield them from the effects of the CS.[6] The standard M40 chemical mask has a silicone rubber face piece with six adjustable straps and a side mount C2A1 canister designed to protect against chemical-biological agents, toxins, and radioactive fallout particles. Individuals immediately feel the effects of the CS if wearing an ill-fitting or defective mask. Army training requires soldiers to break the seal of their protective mask prior to exiting the chamber to demonstrate its effectiveness and instill confidence in its ability to provide protection from chemical threats.[6]

Past research has demonstrated that high temperature (greater than 300°C) dispersal of CS from thermal grenades resulted in the production of at least 23 CS thermal degradation products, some of which are potentially hazardous to human health.[7–10] While military personnel can be exposed to CS generated by thermal grenades during field training exercises and military actions, thermal grenades are not used in mask confidence training.[5] The method used during U.S. Army

mask confidence training disperses CS at a lower temperature than a thermal grenade; however, it is not known what thermal degradation products are generated at these lower dispersal temperatures. Soldiers are potentially exposed to these thermal degradation products through malfunctioning masks or on breaking the seal of their mask as part of their confidence training. Instructors operating the CS chambers are at higher risk. It is unknown whether these thermal degradation products are adequately filtered with the standard Army protective mask because the masks were not specifically tested against CS degradation products.[11]

This research identified the thermal degradation products formed in an Army mask confidence training chamber. Thermal degradation products were also generated and identified under controlled laboratory conditions with temperatures ranging from 150–300°C. The focus of this research was to identify CS thermal degradation products and the temperatures associated with their creation, not to quantify their concentrations.

METHODS

CS Thermal Degradation Products in the Chamber

The chamber in this study (located at the Gunpowder Military Reservation, Glen Arm, Md.) is used by military and law enforcement personnel for training with RCAs. The chamber was 6.8 m long, 3.3 m wide, and 2.6 m high, resulting in an approximate volume of 58 m^3. The chamber was swept, washed with water, and allowed to dry 48 hr prior to sampling.

A CS aerosol generator, constructed and operated in accordance with U.S. Army guidelines, was placed in the center of the chamber floor. The generator consisted of a 387-g coffee can suspended over a Sterno heat source. The can was placed upside down on bricks to form a platform for the CS capsules. The platform had three 1/4-inch holes and five 1/8-inch holes drilled for ventilation (Figure 1).[5]

Solid phase micro extraction (SPME) was used to collect thermal degradation products in this study. When CS is thermally dispersed it is released as an aerosol and as a vapor.[5] In the Kluchinsky et al.[7] study, CS and CS thermal degradation products were collected as aerosols onto filters. Since previous work focused on aerosols, an SPME collection technique that predominantly collects vapors and avoids the complication of using liquid extraction was selected for this research. SPME collection consists of exposing a 1-cm fiber coated with a polymeric material to the sampling environment. Each coating is designed to passively absorb organic vapors from air or organic compounds dissolved in water with varying degrees of collection efficiency.

A 70-μm carbowax/divinyl benzene (CW/DVB) Supelco fiber (Aldrich, Bellefonte, Pa.) was selected for this research after preliminary testing showed CW/DVB collected the most degradation products with the largest GC/MS peaks when compared with CW and poly dimethyl siloxane (PDMS) coatings. CW/DVB combines the absorption properties of CW with high surface area adsorption properties of DVB to help collect a broader array of chemicals with different physicochemical properties.[12] Since aerosols and some highly polar compounds are not readily absorbed by the CW/DVB SPME fiber, the thermal degradation products identified in this research may not be an exhaustive list.

SPME sampling in the chamber consisted of collecting three blank and three thermal degradation product samples per day. An individual wearing the Army M40 series chemical protective mask conducted sampling and remained in the chamber for the duration of sample collection. Blank sampling began by igniting the heat source of the aerosol generator (with no CS added). Three CW/DVB SPME fibers were exposed and placed on 30.5-cm-tall bricks at a 1 m distance from the aerosol generator for 1 min. An exposure time of 1 min was selected because preliminary testing with CS showed that when exposure exceeded 1 min, CS overwhelmed the SPME fiber and the corresponding GC/MS peak made it difficult to detect other compounds. After 1 min of exposure, the SPME fibers were retracted and capped with a silicon septum to prevent sample loss or cross contamination. The sampling blanks were then placed into separate 1-L plastic bags and stored in an ice-filled cooler for transport to the laboratory.

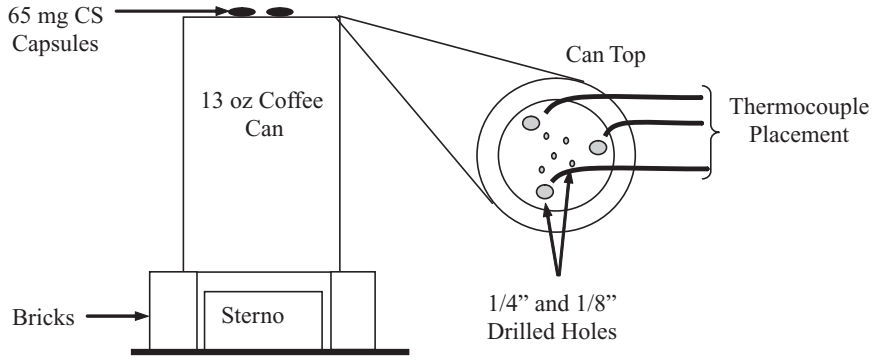

FIGURE 1. US Army CS aerosol generator.

Thermal degradation product sampling began by placing two 65-mg CS capsules (national stock no. 1365-00-690-8556; Defense Technology/Federal Laboratories, Casper, Wyo.) onto the aerosol generator. The dispersal temperature was measured with three thermocouples attached to the surface of the aerosol generator (Figure 1), connected to a Hotmux temperature datalogging system (DDC Corporation, Pennsauken, N.J.). Measurements of the temperature of the platform averaged 257°C (standard deviation 17.3°C).

Approximately 2 min after adding the CS capsules, smoke began to fill the chamber. For this reason, sampling for thermal degradation products began 2 min after the addition of CS capsules. The same SPME technique used to collect the blank samples was used to collect the thermal degradation products. The flame was then extinguished, and the chamber doors were propped open to allow the chamber to clear for at least 24 hr. The sample collection process was repeated on three separate sampling days, resulting in a total of nine blank samples and nine thermal degradation product samples.

All blanks and thermal degradation product samples were analyzed within 1 hr of sample collection using an HP 6890 series gas chromatograph (GC) with a HP-5 (30 m × 0.32 mm ID, 0.25 μm film thickness) column, coupled to an HP 5973 mass spectrometer (MS). The injector port was maintained at 250°C and operated in the split-less mode. Helium was used as the carrier gas at a flow rate of 1.4 mL/min. The GC was programmed to ramp from 40 to 160°C at 10°C per minute; 160 to 172°C at 2°C per minute, and 172 to 300°C at 20°C per minute. The MS transfer line was maintained at 270°C. Mass spectra were collected over the range of 30–250 m/z using electron impact ionization (70eV).

Tube Furnace Experiments

To assess the correlation between temperature and the types of thermal degradation products formed, a ThermoLyne 79500 tube furnace (Barnstead Thermolyne, Dubuque, Iowa) was used to maintain known temperatures. Temperature was monitored via the instrument's digital temperature gauge and verified using the aforementioned Hotmux datalogging system. Pure nitrogen flowed through the tube toward the direction of the SPME fiber collection port at a rate of 475–500 mL/min, which is consistent with tube furnace tests used in previous CS research.[8] Since nitrogen was used in the tube furnace, the absence of oxygen could influence the formation of some oxidation products. However, preliminary studies show close agreement with degradation products between tube furnace experiments and samples taken in open air. Nitrogen was also selected to remain consistent with previous tube furnace experiments involving CS degradation products.[7]

After achieving the desired temperature, a blank sample was collected by inserting an empty combustion boat into the quartz tube using a notched metal rod. After 2 min, a 70-μm CW/DVB SPME fiber was inserted approximately 38 cm from the combustion boat and extended into the nitrogen stream for 1 min. The SPME fiber was then retracted into the protective sleeve and introduced to the GC/MS within 10 sec. The empty combustion boat was then removed using the notched metal rod and replaced with a combustion boat loaded with a 65-mg CS capsule. The CS was given 2 min to disperse, then a 70-μm CW/DVB SPME fiber was inserted into the sampling port and the fiber was exposed for 1 min. The fiber was extended, capped, and analyzed using the aforementioned method. The combustion boat was promptly removed from the tube furnace,

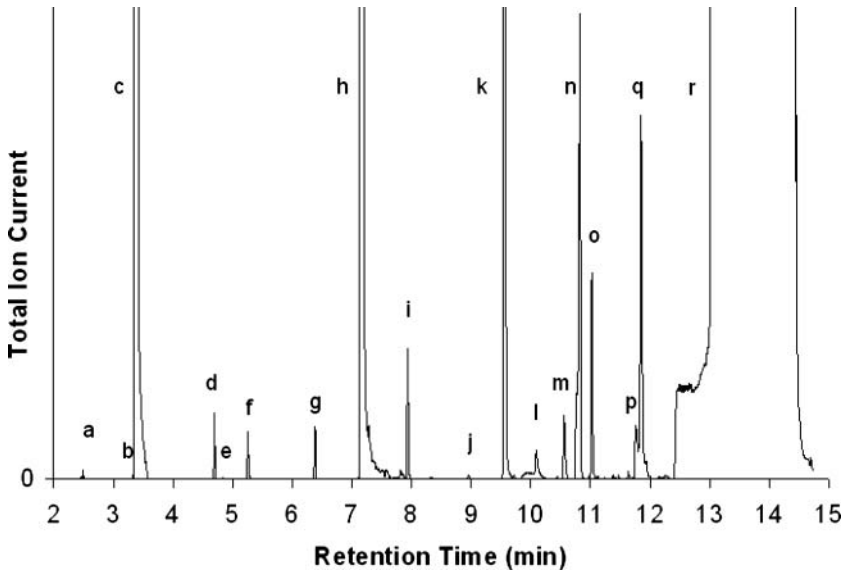

FIGURE 2. GC/MS chromatogram of a CS sample during mask confidence training. a: toluene; b: chlorobenzene; c: malononitrile; d: 2-chlorotoluene; e: benzaldehyde; f: benzonitrile; g: o-chlorostyrene; h: 2-chlorobenzaldehyde; i: 2-chlorobenzonitrile; j: quinoline; k: 2-chlorobenzylcyanide; l: 1, 2-dicyanobenzene; m: 3-(2-chlorophenyl) propynenitrile; n: 4-chloroquinoline; o: 2-chlorohydrocinnamonitrile; p: benzylidene malonitrile; q: 2-chloroquinoline; r: CS.

TABLE I. CS and CS Degradation Product Retention Time and Temperature of Formation

Label	Compound	Retention Time (min)	Current Research Low Temperatures (°C)							Prior Research[A] High Temperatures (°C)			
			150	175	200	225	250	275	300	300	500	700	900
a	Toluene[B,C]	2.51											
b	Chlorobenzene[C]	3.34						X	X				
c	Malononitrile[C]	3.35	X	X	X	X	X	X	X				
d	2 chlorotoluene[C]	4.7							X				
e	Benzaldehyde[C]	4.86							X				
f	Benzonitrile	5.23							X				
g	o-chlorostyrene[C]	6.4		X	X	X	X	X	X				
h	2 chlorobenzaldehyde	7.18		X	X	X	X	X	X	X	X	X	X
i	2 chlorobenzonitrile	7.91				X	X	X	X			X	X
j	Quinoline[C]	8.95						X	X				X
k	2 chlorobenzylcyanide	9.56				X	X	X	X				
l	1,2 dicyanobenzene[B]	10.1										X	X
m	3-(2-chlorophenyl) propynenitrile	10.6						X	X			X	X
n	4 chloroquinoline	10.74						X	X				
o	2 chlorohydrocinnamonitrile	11.05				X	X	X	X				
p	benzylidene malononitrile	11.76				X	X	X	X			X	X
q	2 chloroquinoline	11.86				X	X	X	X				
r	CS[C]	13.2	X	X	X	X	X	X	X	X	X	X	X

Note: X denotes that the chemical was identified at the temperature shown in the column heading.
[A] Kluchinsky et. al.[7] previous high temperature tube furnace experiments using liquid extraction sampling.
[B] Observed only during chamber experiments, not tube furnace experiments.
[C] Compound has an exposure guideline from either OSHA or NIOSH.

and the tube furnace remained at the selected temperature for a subsequent sample. After GC/MS analysis (24:40 min), another combustion boat with a CS capsule was introduced into the system and sampling was repeated.

After one blank and three thermal degradation product samples were collected at the specified temperature, the quartz tube was removed and replaced with a clean tube. The tube furnace was then heated to the next temperature, and the sample and analysis process was repeated. One blank and three samples were collected at 150, 175, 200, 225, 250, 275, and 300°C. All samples were analyzed using the previously described GC/MS settings.

On completion of the tube furnace samples, a 70-μm CW/DVB SPME fiber was exposed to the headspace of an unheated 65-mg CS sample held in a capped 40-mL vial for 1 min. The fiber was analyzed using the previously mentioned GC/MS method within 10 sec. This process was repeated in triplicate to investigate the potential for inadvertently creating degradation products during the analysis process.

RESULTS AND DISCUSSION

CS degradation products were tentatively identified using the onboard National Institute of Standards and Technology (NIST) mass spectra library.[13] Identification was confirmed by comparing retention time and mass spectra of identified peaks to that of known standards analyzed under the same conditions. Replicate samples detected the same thermal degradation products. Figure 2 is a representative chromatogram from the Army mask confidence chamber SPME sampling. No CS thermal degradation products were detected in the blanks, and only trace amounts of 2-chlorobenzaldehyde were found in unheated samples containing pure CS. This compound is formed by the hydrolysis of CS and was also present in unheated CS samples in previous research.[8]

Table I lists compounds identified in the chamber and tube furnace experiments at the temperatures selected in this study (150–300°C). Replicate chamber experiment samples detected the same thermal degradation products. For comparison, Table I also lists the CS degradation products discovered in Kluchinsky et al.[7] that identified degradation products from CS at higher temperatures (300–900°C). The first seven compounds (toluene, chlorobenzene, malononitrile, 2-chlorotoluene, benzaldeyhyde, benzonitrile, and o-chlorostyrene) were identified in this research but were not identified in the Kluchinsky et al. research. These early eluting compounds would have been masked by the analytical method used in the previous study. Kluchinsky et al. collected CS degradation particles on 37-mm PTFE (Teflon) filters, desorbed the thermal degradation products using dichloromethane, and then injected the dichloromethane into the GC/MS. Dichloromethane is an early eluting compound that would mask other compounds

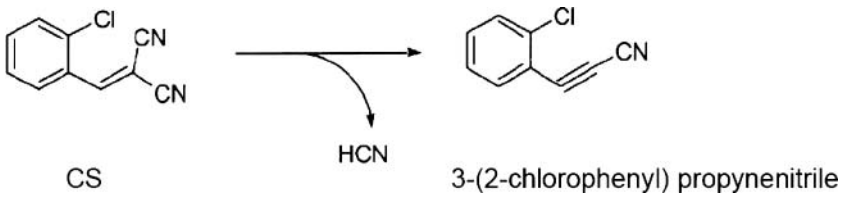

FIGURE 3. Proposed mechanism for the creation of HCN through the thermal degradation of CS[9].

that eluted in the first 8 min of analysis; a solvent delay was used to block the MS from unnecessarily analyzing during this time frame.[7]

Compounds collected via SPME fiber are introduced directly into the GC/MS injection port, eliminating the need for liquid extraction and solvent delays. This SPME method enabled detection of the first seven compounds identified in this research, which may not have been seen by Kluchinsky et al.[7] due to the solvent delay used in their analysis. The CW/DVB SPME fiber, however, predominantly collects vapors, and if degradation products exist only as an aerosol, the SPME collection method would not be as efficient. However, since CS is released as a vapor that condenses to an aerosol after it cools, it is suspected that a large fraction of the degradation products are present both in vapor and aerosol form. The theory that most degradation products exist as both an aerosol and a vapor is supported by the similarity between the previous work that collected aerosols and the current research that collected vapors.

Two later eluting compounds, 2-chloroquinoline and 4-chloroquinoline, were not identified as CS thermal degradation products in the Kluchinsky et al.[7] research. This suggests that these products existed in the vapor phase at the specified temperatures and may not have been captured with the 37-mm PTFE filter used in previous research.

One product of particular interest was 3-(2-chlorophenyl) propynenitrile, which previous research has shown to evolve only at temperatures greater than 500°C.[7] Detection of this compound, which is similar to 2-chloroquinoline and 4-chloroquinoline, suggests that it existed only in the vapor phase at the temperatures specified in this study. This compound is formed through the loss of a cyanide molecule from the parent CS molecule and suggests the presence of hydrogen cyanide (HCN) as shown in Figure 3.[9] HCN presence could not be validated during this work due to the MS scan range used, but the identification of 3-(2-chlorophenyl) propynenitrile at temperatures above 250°C suggests the presence of HCN as a thermal degradation product. Other thermal degradation products identified in this study may also result in the formation of HCN, but this mechanism is the only one documented in peer-reviewed literature.

Toluene and 1, 2 dicyanobenzene were observed in the mask confidence chamber but not in the tube furnace experiments. Past research detected the presence of 1, 2 dicyanobenzene at dispersal temperatures at or above 500°C.[7] It is unknown if toluene would have been detected at or above 500°C because toluene was masked by the solvent delay. These findings suggest that the dispersal method used in the chamber exposes the CS to temperatures at or above 500°C because 1, 2 dicyanobenzene has been detected only at or above 500°C. In this study, 1, 2 dicyanobenzene was not present during the tube furnace experiments that involved well controlled temperatures at or below 300°C.

Observations in the chamber revealed molten CS dripping directly into the flame of the heat source through the ventilation holes, which could have exposed the CS to temperatures at or above 500°C. When the molten CS dripped directly into the flame, the flame grew and produced smoke. The average temperature of the flame was 652°C, measured using the Hotmux datalogging system. CS produces a host of degradation products, including HCN and 1, 2 dicyanobenzene, when dispersed at temperatures of this magnitude.[6–9]

CONCLUSION

Dispersing CS in a mask confidence chamber produces at least 17 unintended thermal degradation products. It cannot be assumed that personnel participating in mask confidence training are adequately protected by the military protective mask, which was not designed to protect against the degradation products identified in this research.[14] Furthermore, the requirement that individuals break the seal of their mask during mask confidence training creates additional potential for exposure.

Human exposure guidelines from the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have been developed for only 7 of the 17 compounds identified in this research.[15] Concentrations of these compounds were not quantified; therefore, a comparison to toxicologic data could not be established. The presence of 3-(2-chlorophenyl) propynenitrile in the mask confidence chamber was confirmed at tube furnace temperatures above 250°C and suggests the presence of HCN.

The current dispersal method appears to disperse CS at temperatures higher than necessary, since CS was observed at temperatures as low as 150°C. Tube furnace experiments showed that the temperature of dispersal and the number of thermal degradation products produced were closely related. At 150°C, CS and only one thermal degradation product were observed, while at 300°C, CS and 15 thermal degradation products were observed. The presence of these 15 compounds

and the additional two compounds believed to evolve at temperatures in excess of 500°C suggests that CS is dispersed at temperatures greater than 300°C in the mask confidence chamber. This theory was supported by visual observation of CS dripping into and dispersing from the flame of the dispersal device (T = 652°C).

RECOMMENDATIONS

The purpose of the aerosol generator is to generate airborne CS; therefore, the ideal aerosol generator should produce airborne CS while minimizing the creation of thermal degradation products. The graded response between the dispersal temperature and the number of degradation products suggests that a CS dispersal device capable of dispersing CS at a lower temperature would be beneficial. It would also be beneficial for the device to inhibit molten CS from dispersing directly from the generator flame, thus minimizing the potential for creation of degradation products.

If use of the current dispersal method continues, further studies should be conducted to quantify the concentrations of the thermal degradation products (vapor and aerosol) and the level of protection provided to the people that are exposed to them. In particular, air sampling should be conducted to quantify the concentration of HCN in the mask confidence chamber, and HCN-specific sampling should be conducted under temperature-controlled conditions to verify the temperature range where HCN begins to form.

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o-Chlorobenzylidene Malononitrile (CS Riot Control Agent) Associated Acute Respiratory Illnesses in a U.S. Army Basic Combat Training Cohort

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ABSTRACT Acute respiratory illnesses (ARIs) are among the leading causes for hospital visits in U.S. military training populations and historically peak during U.S. Army Basic Combat Training (BCT) following mandatory exposure to the riot control agent *o*-chlorobenzylidene malononitrile (CS). This observational prospective cohort studied the association between CS exposures and ARI-related health outcomes in 6,723 U.S. Army recruits attending BCT at Fort Jackson, South Carolina from August 1 to September 25, 2012 by capturing and linking the incidence of ARI before and after the mask confidence chamber to CS exposure data. Recruits had a significantly higher risk (risk ratio = 2.44; 95% confidence interval =1.74, 3.43) of being diagnosed with ARI following exposure to CS compared to the period of training preceding exposure, and incidence of ARI after CS exposure was dependent on the CS exposure concentration ($p = 0.03$). There was a significant pre-/postexposure ARI difference across all CS concentration levels ($p < 0.01$), however, no significant differences were detected among these rate ratios ($p = 0.72$). As CS exposure is positively associated with ARI health outcomes in this population, interventions designed to reduce respiratory exposures could result in decreased hospital burden and lost training time in the U.S. Army BCT population.

INTRODUCTION

Acute respiratory illnesses (ARIs), including the common cold, influenza, pharyngitis, laryngitis, tracheitis, bronchitis, bronchiolitis, pneumonia, and other respiratory ailments, are a global medical concern. Lower respiratory tract infections alone account for over 429 million incident cases per year globally (second only to diarrheal diseases) and lead the world in disease burden.[1] ARIs are a primary contributor to health loss in the United States and are a significant source of morbidity in U.S. military training populations.[2,3] ARIs accounted for more hospital visits and lost work time than any other illness or injury in U.S. military recruits from 2010 to 2011 and were second only to injury and poisoning the following year.[4–6]

The occurrence of ARIs in military recruit populations has been well studied; however, understanding of causal factors is limited.[7–10] A 1998 study of Army Basic Combat Training (BCT) at Fort Jackson, South Carolina found that nearly 50% of recruits sought medical care for ARI-related conditions and over 90% of participants self-reported ARI symptoms. Respiratory-related hospitalizations peaked during training weeks 4 and 5, whereas self-reported febrile illnesses peaked during the third week of training. Investigators attributed the rise in ARI rates to a lapse in availability of an effective adenovirus vaccine.[7] Another study, conducted in 2004, investigated the effect of building design on ARI rates in the BCT population at Fort Jackson, South Carolina. Investigators determined that recruits living in 60-person rooms had a significantly greater ARI risk than those living in 8-person rooms, and that both febrile and afebrile ARI rates peaked during weeks 4 through 6 of the training cycle. Investigators concluded the week of training was significant at almost all levels, across genders, and for both febrile and afebrile ARI outcomes. The authors hypothesized the observed increase in ARI incidence was because of previously unencountered respiratory pathogen exposure, crowded living and training conditions, and decreased immune function because of physical and emotional stress related to entering military training. Immunity increased as the training cycle progressed and trainees adapted to their new environment. This study acknowledged the potential role of training in ARI trends, but did not identify specific training events that may have impacted the reported ARI rates.[11]

In the early 1990s, British Surgeon Lieutenant Commander Pipkin described an ARI outbreak in a Royal Marines training population where cases of influenza peaked shortly following exposure to the riot control agent *o*-chlorobenzylidene malononitrile (CS).[12] CS has a profound effect on the respiratory system, causing immediate pain and irritation in the nose and mouth, excessive nasal discharge and salivation, and sometimes violent coughing spasms, damage to the respiratory epithelium, and pulmonary edema.[13,14] Pipkin speculated that a combination of these effects may have increased influenza incidence within the exposed Royal Marines.[12] The biological plausibility of this hypothesis is reasonable, as opportunistic respiratory infections (including

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those associated with ARI) have been shown to spread via direct and indirect contact, and to commonly occur following chemical irritation or injury.[11,15] Unfortunately, because of a small sample size, Pipkin's hypothesis could not properly be tested, and the question of whether CS exposure can increase ARI rates remains unanswered.

Currently, no ARI studies have considered exposure to CS as a covariate in analyses. All U.S. Army BCT soldiers are exposed to CS in the first 3 weeks of BCT. A 2012 study of over 6,500 soldiers participating in mask confidence training (MCT) during BCT at Fort Jackson, South Carolina showed that unmasked soldiers were exposed to CS concentrations over 125 times the American Conference of Industrial Hygienist Threshold Limit Value Ceiling and over 25 times the National Institute for Occupational Safety and Health Immediately Dangerous to Life and Health (IDLH) level.[16] These limits were established to protect against irreversible health effects and prevent damage to the respiratory epithelium.[17] High levels of acute CS exposure in Army BCT before the observed increase in ARI rates make it temporally plausible that CS exposure experienced during MCT could induce chemical damage and irritation to the respiratory tract. These effects could be diagnosed as ARI or increase susceptibility to respiratory infection, both of which would result in an increase in observed ARI incidence rates. It is also possible that CS-induced expectoration promotes the spread of pathogens responsible for ARIs in this population.

This study examines the association between CS exposure concentrations and ARI health outcomes during Army BCT. The study protocol was approved by the U.S. Army Training and Doctrine Command and the Uniformed Services University of the Health Sciences and was deemed non-human subject research by the Uniformed Services University of the Health Sciences Institutional Review Board because of the observational nature of the study and lack of personal identifying information available to the investigators.

METHODS

This study used an observational, prospective cohort design in a gender integrated cohort of 6,723 exposed soldiers attending U.S. Army BCT at Fort Jackson from August 1 to September 25, 2012 to capture the incidence and distribution of ARI before and after completion of the mandatory MCT portion of their initial military training.

Army training units, designated as approximately 200-person "Companies," scheduled for the MCT were identified by Unit Identification Code through coordination with staff at the chemical, biological, radiological, and nuclear (CBRN) training range. Data on the type of barracks and training week were captured from administrative records provided by Fort Jackson training officials.

Upon arrival to the training site, training units were divided into four ad hoc exposure groups consisting of approximately 50 personnel to proceed through the mask confidence chamber. Exposure group assignment, composition, and size were determined by training officials and were not influenced by investigators.[16] CBRN staff aerosolized 10 CS capsules to establish an initial concentration of CS inside the chamber; the first exposure group entered, conducted a series of exercises, removed their protective masks, and exited the chamber. CBRN staff then aerosolized one additional CS capsule for every 10 people that exited the chamber and the next exposure group entered.[16] This process continued until all four exposure groups completed the training event.

Officials from each training unit used a personnel roster to document trainee attendance, exposure group (1–4), and completion of the chamber exercise. Trainees who completed the MCT with their assigned training unit were enrolled in the cohort; absent soldiers or those who completed the training but were from a different training unit were excluded. Count data specifying the number of trainees that completed the chamber exercise and the number of trainees in each exposure group were provided to the investigators after each training event. CS concentrations were obtained for each exposure group from a concurrent industrial hygiene study.[16] Exposure groups were categorized as one of four exposure categories: 0 to 2 mg/m^3, 2 to 5 mg/m^3, 5 to 10 mg/m^3, and greater than 10 mg/m^3 based on the IDLH value (2.0 mg/m^3) and the incapacitating range (5.0–10.0 mg/m^3) outlined in U.S. Army manuals.[18,19]

Clinically diagnosed and documented inpatient and outpatient ARIs (both febrile and afebrile) were the outcomes of interest. Medical staff queried the Composite Healthcare Computer System for ARI encounters within companies that completed the MCT using the following International Classification of Diseases Version 9 (ICD-9) codes: 079.99 Viral infection, not otherwise specified (NOS); 382.9 Otitis media, NOS; 460 Nasopharyngitis, acute; 461.9 Acute sinusitis; 465.8 Acute upper respiratory infections of other multiple sites; 465.9 Acute upper respiratory infections of unspecified site; 466.0 Bronchitis, acute; 486 Pneumonia, organism NOS; 487.0 Influenza with pneumonia; 487.1 Influenza with respiratory manifestation, not elsewhere classified (NEC); 487.8 Influenza with manifestation, NEC; 490 Bronchitis, NOS; 784.1 Pain, Throat; and 786.2 Cough.

Surveillance count data by training unit were provided by local preventive medicine personnel as part of the existing acute respiratory disease surveillance program.[20] No personal identifying information was provided to the investigators. The surveillance period began 7 days before CS exposure and ended 7 days after exposure (including the day of the exposure). Occurrence of one or more of the ARI-related ICD-9 codes as the primary or secondary diagnosis in a trainee's electronic medical record during the surveillance period was designated as case. This case definition captured both febrile and afebrile ARI cases. Febrile ARI cases had oral temperature of 100.5°F or higher and at least one sign or symptom of acute respiratory tract inflammation (i.e., sore throat, cough, runny nose, chest pain, shortness of breath, headache, tonsillar exudates, or tender cervical lymphadenopathy).[20] All cases not

meeting this definition were categorized as afebrile. Cases were further divided into pre- and postchamber ARI cases. A prechamber ARI case was defined as occurring with a training unit in the 7-day period before CS exposure. A postchamber ARI case was a case that occurred during the 7-day surveillance period beginning with exposure to CS in the mask confidence chamber.

To prevent counting multiple encounters by an individual and ensure the most severe health outcomes were captured, first diagnosis of ARI during the surveillance period was used to establish pre- or postchamber ARI status. However, if a later febrile ARI diagnosis occurred, it took priority and was used to establish pre- or postchamber ARI case status. Those designated as prechamber cases were treated as nonsusceptible for postchamber ARI risk calculations. Cases were cross-referenced with training rosters to determine their cohort and exposure group status. Local preventive medicine personnel provided case counts by training unit, febrile/afebrile, exposure group, and date of encounter.

These counts, along with previously gathered information, were entered into SPSS Statistics for Windows (Version 19, IBM, Chicago, Illinois) for data management. χ^2 analyses, stratified risks, risk ratios (RR), and their 95% confidence intervals (95% CI) were calculated using Open Source Epidemiologic Statistics for Public Health (Version 3.01, www.openepi.com, 2013). SPSS was used to conduct a Poisson regression analysis to examine the relationship between CS exposure concentration and available daily ARI count data allowing the use of ARI diagnoses following CS exposure as variables in the analysis. Power calculations determined a minimum of 64 exposure groups were required to detect a 0.5 difference in risk at 80% power.

RESULTS

There were a total of 6,723 soldiers divided into 134 exposure groups during the surveillance period. All members of the cohort were exposed to CS in the first 3 weeks of training and lived in one of three building types: (1) starship barracks (SS)—fixed facilities consisting of 60-person rooms, (2) relocatable barracks (RL)—movable facilities that can accommodate up to 50 soldiers per room, or (3) rolling pin barracks (RP)—fixed facilities consisting of 8-person rooms. Over half (55.9%) of the cohort completed the mask confidence chamber during their second week of training and most (58.0%) lived in the SS style barracks. Only one training unit consisting of 165 soldiers was housed in the RP style barracks. There were a total of 161 clinically diagnosed cases of ARI in the study population; 47 occurred before CS exposure and 114 after (Table I). Only four (2.48%) of these cases were coded in Composite Healthcare Computer System as febrile ARI cases; all of which occurred postchamber. Figure 1 shows the distribution of postchamber ARI cases by day.

Table II shows the overall risk of developing ARI after exposure to CS was significantly higher than the risk of developing ARI in the surveillance period before completion of the mask confidence chamber (RR = 2.44; 95%CI = 1.74, 3.43). Increased ARI risk was observed regardless of training week the mask confidence chamber was conducted or building the soldier lived in. The Breslow-Day test for interaction of RR over strata did not suggest interaction; stratum specific Mantel–Haenszel adjusted rate ratios were not significantly different than the overall rate ratio suggesting a lack of confounding by chamber week or building type. Overall ARI, prechamber ARI, and postchamber ARI incidence rates were not observed to be different across chamber week ($p = 0.98$, $p = 0.98$, $p = 0.85$, respectively) or building type ($p = 0.18$, $p = 0.72$, $p = 0.17$) (Table II). A χ^2 analysis suggested postexposure ARI cases are dependent on CS exposure concentrations ($p = 0.03$) (Table III). A Poisson regression analysis showed a significant pre-/postchamber ARI difference across all concentrations higher than the referent level (0–2 mg/m^3) ($p = 0.006$); however, no significant differences were detected among these rate ratios ($p = 0.72$) (Figure 2).

TABLE I. ARI Incident Cases by Chamber Week and Building Type

	Prechamber ARI	Postchamber ARI	Total Population
		N (%)	
Overall	47 (0.70)	114 (1.70)	6,723 (100)
Chamber Week			
1	7 (0.66)	20 (1.89)	1,065 (100)
2	26 (0.70)	60 (1.62)	3,693 (100)
3	14 (0.71)	34 (1.73)	1,965 (100)
Building Type			
SS	30 (0.77)	72 (1.86)	3,900 (100)
RL	16 (0.60)	42 (1.58)	2,658 (100)
RP	1 (0.61)	0 (0.00)	165 (100)

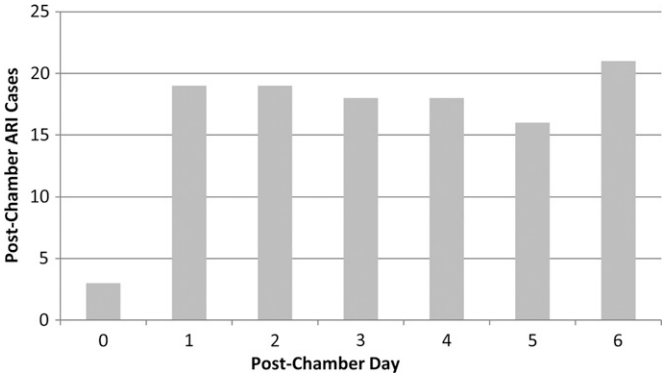

FIGURE 1. Distribution of postchamber ARI by postchamber surveillance day.

DISCUSSION

The results of this study suggest that within our study population, exposure to CS resulted in nearly 2.5 times greater ARI diagnosis risk after MCT compared to the period of training preceding this event. Elevated ARI risk was independent of both the week of training in which CS exposure occurred and barracks building type.

TABLE II. ARI Rates (Per 100 Person-Weeks) by Chamber Week and Building Type

	Prechamber ARI	Postchamber ARI	ARI Incidence	Pre/Post Rate Ratio
	ARI Rates (95% CI)			
Overall	0.70 (0.52, 0.93)	1.71 (1.42, 2.05)	1.20 (1.03, 1.40)	2.44 (1.74, 3.43)
Chamber Week				
1	0.66 (0.29, 1.38)	1.89 (1.21, 2.92)	1.27 (0.86, 1.85)	2.88 (1.22, 6.77)
2	0.70 (0.48, 1.03)	1.64 (1.27, 2.10)	1.17 (0.95, 1.44)	2.32 (1.47, 3.67)
3	0.71 (0.41, 1.20)	1.74 (1.24, 2.43)	1.23 (0.92, 1.63)	2.45 (1.32, 4.54)
Mantel–Haenszel Risk Ratio				2.44 (1.74, 3.43)
Breslow-Day Test for Interaction				$p = 0.91$
Building Type				
SS	0.77 (0.54, 1.10)	1.86 (1.48, 2.34)	1.31 (1.08, 1.59)	2.42 (1.58, 3.69)
RL	0.60 (0.36, 0.98)	1.59 (1.17, 2.15)	1.09 (0.85, 1.41)	2.64 (1.49, 4.69)
RP	0.61 (0.00, 3.70)	0	0.30 (0.00, 1.88)	0
Mantel–Haenszel Risk Ratio				2.44 (1.74, 3.43)
Breslow-Day Test for Interaction				$p = 0.61$

TABLE III. χ^2 Test for Independence of Pre- and Postchamber ARI Cases by CS Concentration

	Prechamber ARI				Postchamber ARI			
Variable	Non-Case	Case	χ^2	p Value	Non-Case	Case	χ^2	p Value
CS Concentration (mg/m^3)			6.60	0.09			8.87	0.03
0–2	131	3			128	3		
2–5	1,852	9			1,832	20		
5–10	2,773	23			2,712	61		
>10	1,920	12			1,890	30		

Over 95% of the cohort was exposed to CS in excess of IDLH (2.0 mg/m^3), with the majority of the population (70.0%) exposed at levels greater than 2.5 times IDLH.[16] The risk of being diagnosed with a postchamber ARI compared to being diagnosed prechamber was significantly elevated in all exposure concentration levels exceeding IDLH. These risks were not statistically different from each other, thus a dose–response relationship could not be established ($p = 0.72$); however, postchamber ARI incidence was dependent on CS exposure concentrations ($p = 0.03$).

The incidence of both prechamber ARI (2.24%; 95%CI = 0.47, 6.81) and postchamber ARI (2.29%; 95%CI = 0.48, 6.81) were elevated in exposure concentrations below IDLH when compared to other groups. However, it is important to note the lack of statistical significance may be because of the sparseness of data at this lowest exposure group ($N = 134$). Also, ARI present in the prechamber (unexposed) population cannot be temporally linked to CS exposure. One possible link could be mixing of the population with CS exposed cases returning to a military unit before it attends the mask confidence chamber, but this was not observed during the conduct of this study.

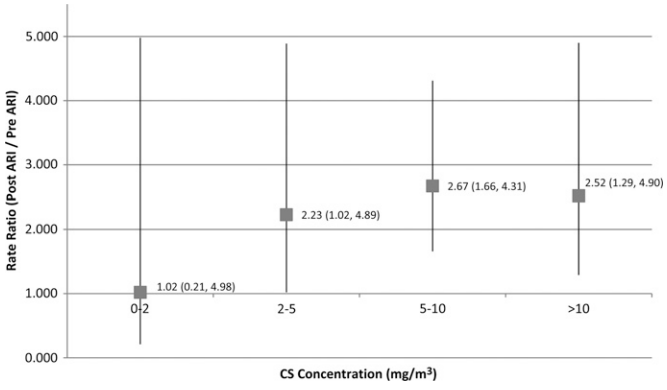

FIGURE 2. Relative incidence of postchamber ARI by CS concentration and 95% CIs.

The increased risk observed at concentrations above IDLH suggests that there may be a threshold concentration in the range of 0.00 to 2.0 mg/m^3 above which promotes symptoms that could result in an ARI diagnosis. It may also suggest that the IDLH value (2.0 mg/m^3) set by National Institute for Occupational Safety and Health is protective against ARI. A decreased risk (RR = 1.02; 95%CI = 0.21, 4.98) was observed at concentrations below IDLH; however, because only 134 (2%) of the entire cohort was exposed at levels below IDLH, it is difficult to determine whether this decreased risk was observed by chance alone. Future studies are needed to better characterize the ARI risk associated with this CS concentration range.

Week of training and living environment have been associated with increased risk of both febrile and afebrile ARI outcomes in a BCT population.[7,11] The results of this study, however, suggest that these covariates do not play a significant role in ARI outcomes during the first 3 weeks of BCT.

Prechamber, postchamber, and overall ARI incidence rates did not vary by week or building type and the pre-/postchamber ARI rate ratio was the same across these strata.

One potential explanation for the lack of significance of these covariates is seasonality. Previous studies were conducted in close proximity to the cold and flu season.[7,11] These populations may have been exposed to a greater number of infectious ARI causing pathogens whose transmission (via direct and/or indirect mechanisms) could be influenced by living and sleeping arrangements. Furthermore, in this scenario, it would take time for infectious ARI to build within a training unit, thus making week of training a relevant factor. This study was well removed from the cold and flu season and observed ARI cumulative incidence of only 2.40% (0.06% febrile, 2.34% afebrile) over the study period.

A more likely reason for the disparity in relevant covariates in this study when compared to previous work is the implementation of a new vaccine in November, 2011, which targeted adenovirus types 4 and 7 and significantly decreased ARI incidence in the BCT populations across all military services.[5,21,22] The impact of the vaccine was evident in this study with only four febrile ARI cases and both febrile and afebrile incidence rates considerably lower than the historic incidence rates.[7,11] The majority of febrile cases (3 of 4) resided in the SS style barracks, which was consistent with previous research.[11] All four cases occurred after completion of the mask confidence chamber at exposure concentrations greater than 2.5 times the IDLH, suggesting that exposure to elevated CS concentrations may increase febrile ARI risk. The low febrile case count, however, could suggest that CS-induced respiratory tract injury rather than infection may have contributed to the number of postchamber ARI diagnoses in this population. However, as symptoms associated with CS exposure are generally short-lived and resolve themselves as time from exposure increases, one would expect CS injury–induced ARI diagnoses to occur immediately following exposure. Our data show that only three (2.6%) postchamber ARI cases were diagnosed the day of the chamber and only 19 (17.7%) were diagnosed the following day. This may suggest that infection rather than CS-induced injury was more prevalent in postchamber ARI diagnoses.

There are several limitations associated with this study. To begin with, it was strictly observational and did not allow for the collection of personal characteristics (e.g., body mass index, prior smoking status, and sex) that have been shown to influence ARI outcomes. Another potential confounder was reuse of protective masks by trainees during MCT. Midway through the study period, investigators witnessed soldiers exit the chamber and transfer their protective mask to waiting soldiers whose originally issued masks were defective. Most masks were exchanged after quickly wiping with one antibacterial wipe; however, some masks were not wiped at all. This practice introduced a potential avenue for the spread of ARI causing pathogens and was not controlled for in the study. Another limitation is that it relied on ARI incidence estimates based on ICD-9 codes in the soldier's electronic medical records, and did not include laboratory-confirmed ARI diagnosis. Without laboratory-confirmed pathogen-specific diagnosis, CS-induced injury of the respiratory tract could have been misdiagnosed as infection. However, the small number of ARI cases on the day of the chamber exposure makes this less likely. In addition, the follow-up period may not have been long enough to identify these misdiagnosed cases or other afebrile ARI that may have progressed to febrile ARI later in the training cycle. A combination of these factors makes it difficult to determine whether the cases captured in this study are a result of infection or injury. Finally, exposure concentrations were assigned based on an area sampling methodology that assumed that CS was evenly dispersed in the mask confidence chamber.[16] Although this is considered an acceptable method for estimating exposures, it is not as precise as individual monitoring and could have impacted the results observed here.

CONCLUSION

This is the first study to consider CS exposure as potential risk factor for ARI diagnoses in a BCT population. Regardless of the cause for diagnosis (injury or infection), ARIs have a significant impact on the health care system and on the readiness of today's fighting force. This study showed that those exposed to CS in the mask confidence chamber had nearly 2.5 times greater risk of being diagnosed with ARI after completion of this training event. It also suggests that postexposure ARIs are dependent on the CS exposure concentration. As ARI is positively associated with CS exposure in this population, interventions designed to reduce or eliminate the exposure could result in decreased hospital burden, health care costs, and lost training time within the BCT population. It is also possible that this study could have broader implications to other military populations and law enforcement personnel.

Preliminary results of this study were provided to medical and training officials at Training and Doctrine Command, through the Army Public Health Command, resulting in an Army-wide intervention by the U.S. Army Safety Office targeting CS exposure levels to mitigate the risks reported here. This intervention, All Army Activities message 051/2013, was implemented in March 2013 mandating lower CS concentrations, shorter exposure times, semiannual industrial hygiene surveys, and periodic wet cleaning of all Army mask confidence chambers.[23] Ongoing research is being conducted at the Uniformed Services University of the Health Sciences to determine the efficacy of this intervention in lowering CS exposure concentrations and mitigating the risks reported here. Future research is required to determine intervention impact on a soldier's perception of the protective nature of their assigned CBRN protective equipment and to determine if this intervention reduced lost training time, health care costs, and hospital burden.

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Tear gas: an epidemiological and mechanistic reassessment

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Deployments of tear gas and pepper spray have rapidly increased worldwide. Large amounts of tear gas have been used in densely populated cities, including Cairo, Istanbul, Rio de Janeiro, Manama (Bahrain), and Hong Kong. In the United States, tear gas was used extensively during recent riots in Ferguson, Missouri. Whereas tear gas deployment systems have rapidly improved—with aerial drone systems tested and requested by law enforcement—epidemiological and mechanistic research have lagged behind and have received little attention. Case studies and recent epidemiological studies revealed that tear gas agents can cause lung, cutaneous, and ocular injuries, with individuals affected by chronic morbidities at high risk for complications. Mechanistic studies identified the ion channels TRPV1 and TRPA1 as targets of capsaicin in pepper spray, and of the tear gas agents chloroacetophenone, CS, and CR. TRPV1 and TRPA1 localize to pain-sensing peripheral sensory neurons and have been linked to acute and chronic pain, cough, asthma, lung injury, dermatitis, itch, and neurodegeneration. In animal models, transient receptor potential inhibitors show promising effects as potential countermeasures against tear gas injuries. On the basis of the available data, a reassessment of the health risks of tear gas exposures in the civilian population is advised, and development of new countermeasures is proposed.

Keywords: tear gas; pepper spray; capsaicin; chlorobenzalmalononitrile; CS; CN; CR; TRPV1; TRPA1

Tear gas agents for riot control

Over the past several decades, tear gas has been used as a common riot-control agent (RCA) by law enforcement to quell protests, riots, and civil unrest. Tear gas use has dramatically increased in recent years, with very large amounts released in population centers in Turkey,[1] the United States,[2] Hong Kong,[3] Greece,[4] Brazil,[5] Egypt, and Bahrain.[6,7]

Tear gas is generally perceived to be a sublethal incapacitant.[8] A 2003 analysis of several tear gases and incapacitants concluded that, on the basis of available toxicological evidence, commonly used tear gases have a large safety margin for life-threatening or irreversible toxic effects.[9] Another medical review published in 2013 concluded that, in the majority of exposures, significant clinical effects are not anticipated.[10] However, there are debates surrounding the acceptability of tear gas use for riot-control purposes, especially in the background of the recent massive use. Many believe the risks of tear gas exposure are understated and that perceived risks are based on insufficient human epidemiological and mechanistic data.

The major RCAs used since World War II include o-chlorobenzylidene malononitrile (CS), oleoresin capsicum (OC, pepper spray), dibenz [*b*,*f*]-1,4-oxazepine (CR), and 1-chloroacetophenone (CN)[11] (Fig. 1). The most commonly used RCA until the 1950s was CN, but a search for an alternative to CN was initiated in response to dissatisfaction with the potency and stability of the compound.[12] CS was discovered by two American scientists in 1928[13] but was only developed for use as an RCA decades later.[14] This compound—considered to be more potent but

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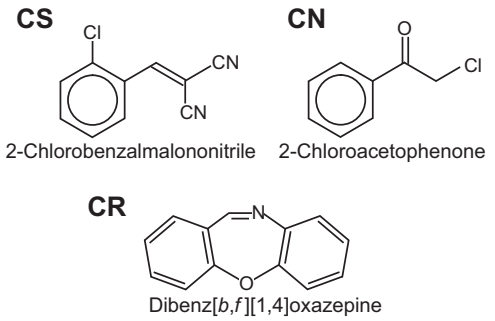

Figure 1. Chemical structures of commonly used tear gas agents o-chlorobenzylidene malononitrile (CS), 1-chloroacetophenone (CN), and dibenz[*b*,*f*]-1,4-oxazepine (CR).

less toxic than CN at that time—was adopted as the standard RCA of the U.S. Army in 1959.[12,15] In the following decades, CS became the most common RCA, and it is now used widely.[16] CS, CN, and CR tear gas agents are electrophilic agents, and their structures are presented in Figure 1.

OC is a mixture of several compounds extracted from chili peppers, with capsaicin as the major active ingredient.[14] Pepper spray was developed as an animal repellent in the 1960s, but law enforcement agencies in the United States began using the compound for personal protection in the 1980s and 1990s.[11] The rise in popularity of OC was similar to the rise in popularity of CS: OC was adopted as a less dangerous alternative to Mace, an aerosol self-defense spray whose primary component was CN.[11] CS and OC are now almost exclusively used as the RCAs of choice. The use of tear gas agents in warfare between military forces is banned under the 1993 International Chemical Weapons Convention, Geneva (Organisation for the Prohibition of Chemical Weapons), likely due to fear of escalation of chemical warfare. Some countries, including the United States, issued executive orders permitting the use of tear gas by military forces against rioting civilians and nonmilitary combatants and for troop extraction.[17] The domestic use of tear gas agents is not covered by the Geneva Convention.[17] Lack of epidemiological and mechanistic data on the spectrum of health effects of tear gas hinders the development of treatment plans and countermeasures and the medical understanding of long-term effects. In this review, we summarize the existing epidemiological data on the health effects and biological mechanistic effects of tear gas agents and make recommendations to bridge the paucity of knowledge in this area.

Deployment technologies

Though commonly referred to as "tear gas," the active compounds are not actually gases but solids. RCAs are deployed in many different ways, as personal defense sprays or from grenades or canisters.[12] Sprays use a liquid formulation that is released from a pressurized dispenser, while grenades and canisters use a powdered form blended with a pyrotechnic mixture that can be aerosolized for dispersion as a smoke or fog.[12] A common solvent for sprays is methyl isobutyl ketone (hexone), which is also considered hazardous.[18,19] CS tear gas agent is typically aerosolized as 3- to 10-μm microencapsulated microparticles in aerosols. The typical pyrotechnic composition for dissemination of CS riot control agent consists of 45% CS agent, 30% potassium chlorate, 14% epoxy resin, 7% maleic anhydride, 3% methyl nadic anhydride, and 0.03% mixed residual balance.[20] Although the intrinsic toxicities of these ingredients in the pyrotechnic composition were not studied in detail, their safety data sheets show significant toxicities. These pyrotechnic devices can be thrown by hand or fired from launchers, engaging targets up to 400 m away and penetrating window glass,[12] with dispersion area ranges from 60 to 300 m^2.[21] Aircraft- and vehicle-mounted dispersers can be used for RCA deployment, and aerial drone–based RCA deployment systems have been tested and requested by several law enforcement agencies, with widespread adoption likely once the technology has sufficiently advanced.[12]

Pepper spray (OC) is typically dispersed from a handheld canister.[21] However, it is also available in a number of different types of grenades and projectiles.[22] CN can be dissolved in a solvent, used in irritant sprays like Mace, or deployed from thermal grenades.[11]

Health effects of tear gas exposures

Exposure to tear gas agents produces a wide spectrum of health effects, including acute and chronic effects. Studies have demonstrated specific receptor-mediated mechanisms of action of tear gas agents.[23,24] Whereas these specific receptors contribute to their acute painful and irritant effects, the electrophilic reactivity of the agents, together with the toxicities of solvents and pyrotechnic

reaction products, engages multiple toxicological mechanisms that remain to be studied.

Immediate exposure effects

CS and OC produce similar symptoms. Acute CS exposure at concentrations generally used by law enforcement for riot-control purposes results in instantaneous irritation to the eyes, nose, mouth, skin, and respiratory tract.[9] Dermal effects include itching, stinging, and redness, with potential blistering and allergic contact dermatitis.[25] Ocular exposure can result in lacrimation, blepharospasm, itching, and burning sensation.[26] When inhaled, CS often leads to coughing, choking, salivation, and chest tightness.[11] OC exposure causes pain and tingling in the respiratory tract, accompanied by coughing.[11] Signs and symptoms of OC contact with eyes include lacrimation, inflammation of the conjunctiva, blepharospasm, redness, pain, burning, and edema.[14] Some evidence indicates that OC may also temporarily inhibit the blink reflex and limit responsiveness to mechanical and chemical stimuli to the eye.[14] Like CS, dermal effects of OC can include pain, tingling, redness, swelling, and blistering.[14] The effects of CN are similar to those of CS and OC,[27] but are significantly more severe and potentially life threatening. CN is a more toxic lacrimator than CS and is more likely to cause serious injury to the skin.[11]

Respiratory effects

Much of the research surrounding the effects of tear gas exposure was derived from laboratory animal research or from small studies of previously healthy individuals in controlled conditions, and several believe this level of research is inadequate for safety assessment.[17,28] For example, one study concluding that tear gas exposure was not associated with increased airway resistance was conducted on a sample of only seven healthy military volunteers, and those with a history of chronic respiratory illness were excluded.[16] Tear gas use in riots or instances of large-scale civil disorder could result in extended, repeated, or highly concentrated exposures, which pose a greater threat to respiratory health.[14,29] High concentrations of CS or OC can cause severe respiratory symptoms, such as reactive airways dysfunction syndrome, in an individual exposed to both CS and OC and hemoptysis.[30–32] Capsaicin infiltration of the lower respiratory tract can induce pulmonary edema, apnea, and respiratory arrest.[11] Surveys performed after recent massive-scale tear gas deployments in Turkey reported persistent cough, chest pain, sputum production, hemoptysis, breathing difficulties, and nasal discharge, sometimes lasting for weeks after exposure.[28] Lung function tests observed restriction and medium and small airway obstruction that was more severe in women.[22] Respiratory effects were also observed in residents of the areas where tear gas was deployed, suggesting that tear gas agents represent a persistent environmental health hazard.[29]

Another Turkish study with 93 males frequently exposed to tear gas and 55 unexposed subjects found that tear gas–exposed subjects were at greater risk for chronic bronchitis.[33] A study of 34 young adults exposed to CS in a confined space during a confrontation with the police reported no long-term sequelae.[34]

CS-induced respiratory illness during military training

Unexpected respiratory risks linked to tear gas exposures were discovered in epidemiological studies by the U.S. Army, analyzing health effects in more than 6000 army recruits exposed to CS in chambers during gas mask–confidence training. This relatively young and healthy population developed a high risk of presenting with acute respiratory illness in the time after CS exposure, with increasing risk at higher exposure concentrations.[35,36] Exposure levels considered for many years safe and necessary for training were determined to far exceed the National Institute for Occupational and Safety and Health and Occupational Safety and Health Administration safety levels.[37] These findings led to immediate measures limiting exposure concentrations and times, improving decontamination procedures, and imposing frequent hygiene and health monitoring. Measures of respiratory illness included throat pain, cough, bronchitis, nasopharyngitis, sinusitis, and other indications. CS exposures were also associated with an increase in respiratory infections, including influenza. Follow-up studies demonstrated that lowering CS exposures during training effectively reduced the risk for respiratory illness.[38] Whether these lower concentrations are also safe for diverse civilian populations remains unclear. Follow-up epidemiological studies in military populations would represent a unique opportunity to

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identify potential long-term health effects of tear gas exposures.

Ocular effects

Tear gas deployed at close range can cause severe ocular injuries, including corneal stromal edema, conjunctival tearing, and deep vascularization of the eye.[39] Other ocular complications include vitreous hemorrhage, traumatic optic neuropathy, symblepharon, pseudopterygium, infective keratitis, trophic keratopathy, glaucoma, and cataracts.[39] One report described four subjects who developed corneal erosion following exposure to pepper spray, indicating that OC or a solvent in the spray may cause nerve damage.[40]

Skin burns and dermatitis

Physicians examining CS-exposed patients often report skin burns, especially when large quantities are used, as in a case involving a riot at a Vietnamese refugee detention center in Hong Kong.[30,32] Multiple cases of unusually severe skin reactions in response to CS exposure have been reported, including severe facial erythema and swelling that obscured vision.[41] Physicians from the Department of Dermatology at San Francisco General Hospital observed severe CS-induced erythematous dermatitis of the face, neck, and hands.[42] Cases of allergic contact sensitization were reported with erythematous patches and multiple vesicular eruptions on the skin following heavy exposure to CS.[43] Ninety percent of workers in a plant manufacturing a CS agent reported a history of dermatitis on the arms and neck, with 7% showing positive patch-test reactions to CS, suggesting that CS may act as a contact sensitizer.[44]

Cardiovascular and gastrointestinal effects

Irritation of the gastrointestinal tract due to ingestion of compounds like CS may cause nausea, vomiting, diarrhea, and hematemesis.[14,32] Various cardiovascular effects, including tachycardia and transient hypertension, have been observed in some individuals, likely initiated by sensory–autonomic reflexes or anxiety, pain, or psychological distress.[45]

Severe injuries and deaths

There have been numerous case reports of injuries and fatalities associated with exposure to high concentrations of tear gas or exposure in enclosed spaces or for extended periods of time. Deaths and respiratory tract injuries were reported after release of tear gas in prisons.[46–48] CS and OC are increasingly used in prison systems, often in enclosed and poorly ventilated spaces. Deaths of inmates with preexisting respiratory conditions have been linked to multiple CS and OC exposures and lack of decontamination.[49] Other studies documented cases of death within 1 h of exposure to OC, though a direct causal link has not yet been established.[14] Severe injuries and deaths have been reported during the massive-scale deployments of tear gas munitions in Egypt, Turkey, Bahrain, and Brazil. These were often caused by direct or close impact of tear gas munitions causing severe head and eye injuries and burns.[50,51] A well-documented case is the death of 37 Egyptian inmates in a prisoner van into which tear gas munitions were fired.[52] Circumstantial reports suggest a correlation between CS exposure and miscarriage.[16,51,53]

Lack of epidemiological research in tear gas–exposed civilian populations and high-risk groups

There is a significant amount of research examining the acute effects of RCA exposure among small samples of healthy individuals in controlled conditions, but little information has been gathered on the consequences of exposure in the field. A review on exposure to the CS tear gas agent attempted to compile case reports on the basis of PubMed and Scopus literature searches.[54] The real-world conditions in which tear gas is used make it difficult to discriminate between the effects of different RCAs and to conduct effective epidemiological investigations.[16] It is often not possible to ascertain the exposure concentration and duration among exposed individuals, and weather or terrain factors can further complicate analysis. Because of the difficulties associated with conducting epidemiological investigations of RCA effects and the lack of public support for these studies, few epidemiological studies have been published, and the reliability of the results is often deficient. The situation is further complicated by the fact that research conducted by various military organizations is often classified,[55] and organizations may be denied access to health information during instances of civil unrest.[56]

While prolonged exposure can lead to increased severity of symptoms, conclusions from past research indicated that most effects should resolve within minutes of removal from exposure. However,

evidence supporting this conclusion came from significantly limited studies. For example, one oft-cited study deemed CS tear gas safe on the basis of outcomes of controlled exposures of 35 healthy male volunteers, without considering the effects on children, women, the elderly, or subjects affected by preexisting conditions.[26] Deficiencies in the currently available research have impeded understanding of all of the risks potentially associated with chemical RCAs. The effects of RCA exposure among sensitive populations and among those with underlying health conditions are one such area where the level of risk is unclear. Individuals suffering from asthma or reactive airways disease could be at greater risk for more serious adverse effects from tear gas exposure, as chemical RCAs cause significant respiratory symptoms, which could plausibly be exacerbated in the presence of underlying respiratory illness. Current research on the issue, however, remains equivocal on the topic. A study of CS exposure in one group found no increase in airway resistance after exposure, but the study subjects only included healthy volunteers, and those with a history of asthma were excluded from the study.[16] A report of CS exposure in a nightclub indicated that patients with asthma experienced no greater sensitivity to the RCA,[57] and similar results were published in the report of an inquiry into a CS exposure in Londonderry in 1969.[58] However, according to a study of RCA exposure in South Korea, physicians reported that patients with asthma and chronic obstructive pulmonary disease experienced deterioration of lung function following tear gas exposure—some to a serious degree necessitating a longer stay at the hospital.[16]

Other populations besides those with underlying respiratory conditions may also be at a greater susceptibility to harm from RCAs. The British Department of Health and other sources reported that individuals with hypertension or cardiovascular disease, as well as those taking neuroleptic medications, may be more susceptible to CS, calling for more research to be done in these populations.[11,59] In one case study, a 40-year-old male was diagnosed with acute myocardial infarction (AMI) following exposure to pepper spray, indicating that OC could potentially be a triggering factor.[60] There is a strong relationship between inhalation of particulate matter and AMI.[61] While the acute pain and cardiorespiratory distress following exposure can contribute to triggering AMI, the consequences of inhalation of microencapsulated and precipitated particles, oil droplets, and particles generated during combustion from tear gas sprays or munitions need to be further investigated.

Biological targets and mechanisms

TRPV1: the target of capsaicin in pepper spray

The active noxious agent in pepper spray is capsaicin, purified and enriched from pungent chili peppers. The molecular target of capsaicin, TRPV1, was discovered in 1997.[62] TRPV1 is a transient receptor potential (TRP) ion channel expressed in nociceptors, the pain-sensing peripheral sensory nerves of the trigeminal, vagal, and dorsal root ganglia (DRG). Nociceptor nerve endings are present in all organs and the body surface, including the skin, cornea, conjunctiva, and the mucous membranes of the upper and lower airways and lung. TRPV1 is a nonselective cation channel that, when activated by capsaicin, promotes neuronal depolarization. TRPV1 is also activated when nerve endings are exposed to noxious heat, acting as a thermal warning sensor for imminent tissue damage. Tissue acidification or acid exposures lead to sensitization or activation of TRPV1. TRPV1 is sensitized through signaling from a range of G protein–coupled receptors and receptor tyrosine kinases activated during injury and inflammation. These include the bradykinin receptor, prostaglandin receptors, nerve growth factor receptors, and cytokine and chemokine receptors.

TRPA1: the reactive irritant receptor mediating the acute effects of tear gas agents

The tear gas agents CS, CN, and CR are structurally dissimilar, suggesting they might bind to different targets (Fig. 1). However, a single target, TRPA1, was identified as mediating the acute noxious effects of these agents and of many similarly acting chemical exposures.[24,63] TRPA1 is also a TRP ion channel and, similar to TRPV1, is expressed in nociceptors. Pain neurobiological studies initially revealed that TRPA1 is the target of mustard oil (allyl isothiocyanate), the pain-inducing and lachrymatory product in mustard, wasabi, and horseradish.[64] Together with capsaicin, mustard oil was used as an important chemical tool to characterize the function of nociceptor subtypes in pain transduction.

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Mustard oil is an electrophile, and TRPA1 was also found to be responsive to similar naturally occurring isothiocyanates and related compounds in onions and garlic.[64,65] Isocyanates, such as mustard oil, are electrophiles thought to act as defensive agents of the plants against herbivores. Mustard oil is not to be mistaken with sulfur mustard and nitrogen mustard, the blistering agents belonging to a different class of chemical warfare agents in mustard gas. While these agents share a similar odor with mustard oil, mustard gas exposure is not immediately painful and has delayed effects.

Intriguingly, pretreatment of animals with capsaicin desensitized them to perceiving pain from mustard oil, suggesting that receptors for these agents may be expressed in the same nerve fibers, where they desensitize each other. Indeed, TRPV1 and TRPA1 were found to be expressed in the same population of nociceptors. Toxicological studies have shown that capsaicin pretreatment desensitizes neuronal responses to a wide range of chemical sensory irritants targeting nociceptors. One example is the volatile electrophile acrolein, an unsaturated aldehyde and the major airborne irritant in smoke from fires and combusted tobacco and in diesel exhaust.[66] Acrolein was used briefly as an irritant gas in warfare in World War I. Capsaicin pretreatment was shown to render mouse nasal trigeminal neurons unresponsive to airborne acrolein.[66] Studies in heterologous expression systems revealed that both rodent and human TRPA1 channels were activated by acrolein.[23] Similar effects were seen with croton aldehyde, another tobacco smoke aldehyde, and even saturated aldehydes, such as formaldehyde and acetaldehyde.

The discovery of TRPA1 as an electrophilic irritant receptor inspired additional studies that in 2008 identified TRPA1 as the principal target of the tear gas agents CN, CS, and CR, *in vitro* and *in vivo*.[24,63] These three agents are among the most potent TRPA1 agonists known, with CS and CR activating human TRPA1 channels in the low nanomolar or subnanomolar range, more than 10,000-fold more potent than mustard oil and other natural TRPA1 agonists. Modified electrophilic CR-based chemicals were even more potent than the parent compound.[67] Mice with a targeted deletion in *Trpa1* displayed no or only minimal acute pain behavior when exposed to CN or CS, confirming the essential role of TRPA1 in their sensory detection.[24] In human studies, the potency of tear gas agent derivatives toward TRPA1 showed clear correlation with their perceived irritancy, suggesting that TRPA1 also contributes to tear gas sensing in humans.[68]

TRPA1 is activated by a large variety of structurally unrelated irritant chemicals.[69] This sensitivity to multiple chemicals cannot be explained by a traditional pharmacological ligand–receptor model. Biochemical studies revealed a reactivity-based activation mechanism of TRPA1, in which electrophilic and oxidizing activators modify cysteine residues in the N-terminal domain of TRPA1, resulting in covalent modification leading to channel activation.[70] Thus, TRPA1 can be considered a peripheral neuronal reactivity detector, signaling the danger of imminent injury by electrophilic or oxidant exposures.

Health effects related to TRP channel activation

The discovery of TRP ion channels as the primary sensory detectors for environmental, chemical, and physical stimuli in peripheral sensory neurons was a watershed in sensory neurobiology and pharmacology. While initial studies focused on the roles of TRPV1 and TRPA1 in pain, more recent discoveries revealed that these ion channels play fundamental roles in reflex responses in the respiratory, cardiovascular, digestive, and other organ systems and in acute and chronic inflammatory and degenerative conditions. These pathological mechanisms need to be taken into consideration when reassessing the acute and chronic health effects of tear gas exposures, especially in exposed individuals affected by chronic health conditions within a diverse civilian population.

Pain

Tear gas and pepper spray exposures cause immediate and severe pain leading to incapacitation. Ocular and nasal pain are sensed almost immediately and are initiated by activation of trigeminal nerve endings in the cornea and nasal passages that are highly sensitive to chemical exposures. Since TRPV1 and TRPA1 were identified as major pain-initiating receptors, the pharmaceutical industry has developed a wide range of inhibitors for development as analgesics. Analgesic action was demonstrated in animal models of acute and inflammatory pain. In clinical trials, TRPV1 inhibitors alleviated

heat-induced pain, with moderate effects toward other pain modalities. The development of TRPA1 inhibitors began later, with clinical trials ongoing at this time.

Cough and airway obstruction

Cough is elicited when a chemical irritant activates vagal sensory nerve endings in the larynx. Vagal sensory nerves express higher levels of TRPV1 and TRPA1 than the trigeminal ganglia and DRG. Capsaicin is often used as a cough stimulus in clinical settings, and many airborne TRPA1 agonists are cough triggers. Tear gas and pepper spray exposures trigger cough directly but also cause profuse secretions within the airways due to sensory–autonomic reflexes. Secretions further aggravate cough and contribute to incapacitation by obstructing normal breathing and eliciting the fear of suffocation. Recent studies have implicated TRPA1 sensitization and heightened activity in chronic cough and cough hypersensitivity.[71]

Asthma

Recent studies in animal models of allergen-induced asthma have shown that TRPA1 plays a critical role in the initiation and maintenance of asthmatic inflammation, airway hyperreactivity, and smooth muscle contraction.[72,73] Human genetic studies have associated polymorphisms in *TRPA1* with reduced control of asthma.[74] Irritant-induced asthma, manifesting as airway hyperreactivity following irritant inhalation, was also shown to depend on TRPA1.[75] With an asthma prevalence of 8.4% in the U.S. population and similar levels around the world, there is a high chance of exposure to tear gas and the development of complications in asthmatics. Indeed, the most severe complications reported after tear gas deployment involve asthma attacks.[34]

Chronic obstructive pulmonary disorder and lung injury

TRPA1 is activated by many of the principal irritants in tobacco smoke, including acrolein, crotonaldehyde, and smoke particulates.[23,74] In chronic smokers, inhalation of these irritants contributes to the etiology of chronic obstructive pulmonary disorder. TRPA1 has also been implicated in ventilator-induced lung injury, in which mechanical stress and oxygen activate sensory neurons that may contribute to the observed inflammatory response. Activation of TRPA1 in the lung was shown to trigger the release of proinflammatory neuropeptides, such as CGRP, substance P, and neurokinin A.[24] While additional research is needed to delineate the role of TRPA1 in lung injury, these findings suggest that TRPA1 activation may aggravate preexisting pulmonary inflammation, injury, and remodeling processes in smokers and other affected individuals.

Cardiac arrhythmia

Irritant exposures have been linked to cardiovascular stress and sensory–autonomic dysregulation of cardiovascular function. Studies in rats prone to arrhythmia have shown that respiratory exposures to diesel exhaust or acrolein strongly increase the risk of arrhythmia through sympathetic activation.[76,77] Rats treated with a TRPA1 inhibitor were resistant to exposure-induced arrhythmias, suggesting that TRPA1 is a key chemical detector triggering circuits that alter cardiovascular control.[76,77] Tear gas exposures may have similar effects in humans, suggesting that exposed individuals with preexisting cardiovascular conditions and arrhythmias may be at increased risk of developing cardiovascular complications.

Dermatitis and itch

TRPA1 agonists, such as mustard oil, are known to cause skin inflammation and edema that are diminished in TRPA1-deficient mice. TRPA1 plays a key role in the neuronal control of skin inflammation and in the neuronal transduction of itch signals mediated by a specialized subpopulation of sensory neurons.[78] TRPA1 is coupled to pruritogen receptors and is essential for full development of inflammation and itch in hapten-induced contact dermatitis.[78] Tear gas exposures of the skin cause pain, skin edema, and inflammation with itching. Tear gas agents can likely act as haptens themselves, and skin hypersensitivity reactions to tear gas agents, including allergic contact dermatitis, have been reported.[34,43]

Thus, atopic and exposed individuals affected by contact dermatitis may be at risk of developing adverse skin reactions. The appearance of clinical cutaneous symptoms varies widely from a few minutes to several weeks. A summary of dermal clinical symptoms following CS tear gas exposure and latency periods has been published.[54]

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Peripheral nerve damage

The analgesic properties of TRPA1 inhibitors are currently being tested in clinical trials in patients affected by diabetic neuropathy, a painful neurodegenerative condition. TRPA1 plays a key role in the peripheral neuronal hypersensitivity to metabolic stress in diabetic animal models.[79] Chronic TRPA1 activity leads to nerve calcium overload and excitotoxicity, resulting in chronic pain signaling and peripheral neurodegeneration, leading to loss of sensation or altered sensations. Other chemically induced neurodegenerative conditions were also linked to TRPA1, including chemotherapy-induced neuropathies.[80] With more than 9% of the U.S. population affected by diabetes and diabetes rates approaching similar levels in other countries, this population needs to be considered at risk during tear gas exposures. Ligands of TRPV1 and TRPA1, when applied topically, are known to cause extensive desensitization and remodeling of cutaneous nerve endings. With tear gas agents being so highly potent, it is likely that similar effects occur. While desensitized nerve endings can recover and normal sensory capacity can be reestablished, high-level exposures and local contamination may cause long-term damage to the underlying sensory innervation.

TRP channel inhibitors as countermeasures against tear gas effects

At this time, no mechanism-based countermeasures are available to alleviate the noxious effects of tear gas and pepper spray exposures. Countermeasures mostly involve decontamination strategies, including rinsing with water and buffered solutions, discarding contaminated clothing, and medical supportive treatment.

Highly potent and selective TRPV1 inhibitors have been developed and tested in animal studies and in clinical trials with proven efficacies for capsaicin-induced and thermally induced pain.[81] It remains unclear whether these inhibitors will be developed further toward U.S. Food and Drug Administration approval for pain indications and will be considered for testing as countermeasures against pepper spray exposures.

A TRPA1 inhibitor was efficacious for prevention of tear gas agent (CN and CS) exposure–induced ocular pain in mice.[24] Since anti-inflammatory and analgesic effects have been observed for TRPA1 inhibitors in multiple models of chemical injury and inflammation, it is highly likely that TRPA1 inhibitors will alleviate at least some of the tear gas–induced effects. There remains concern about the specificity and poor solubility of the tool compounds available for TRPA1 inhibition. The conclusion of clinical trials using TRPA1 inhibitors in diabetic neuropathic patients is eagerly awaited with the hope that more advanced inhibitors will be made available for testing in other conditions, including irritant and tear gas agent exposures.

Other targets of tear gas agents

Owing to their electrophilic properties, tear gas agents likely react with many other biomolecules in the eyes, respiratory tract, and skin. The nature of these targets is largely unknown. Similar to acrolein and related electrophiles, tear gas agents may damage and deplete biological redox systems in the lining fluids of epithelia and within cells and mitochondria, modify structural proteins and nucleic acids, and inactivate enzymes. There has been minimal research on endocrine effects, immunologic consequences, and histological changes from CS exposure, but some animal studies indicate that potential effects may occur. Studies in rats injected with CS found histological changes in the adrenal gland and the thyroid, though it is not clear whether dermal or inhalational exposure to CS in humans would result in a similar response.[82] Another study of CS exposure in mice reported suppression of the humoral immune response and elevated corticosteroid levels.[83] The mutagenicity and potential carcinogenic effects of RCAs are also not well understood, with research clearly lacking. Some laboratory studies indicate that CS is not mutagenic[84] or is weakly mutagenic,[85] but the results of carcinogenicity studies have not been confirmed,[86] and much of the research has been limited to laboratory studies rather than human studies. The mutagenicity of capsaicinoids has been tested extensively, but the results have been conflicting.[14] Dispersal of RCAs involving pyrotechnic mixtures can produce thermal degradation by-products that could potentially be dangerous to human health.[87]

Discussion and recommendations

The decision by law enforcement to use tear gas during civil disorder is understandable, as CN, CS, and OC are effective RCAs and reduce the risk of injury to law enforcement personnel and demonstrators

when used instead of physical force.[10,88] However, the massive increase in tear gas deployments worldwide, accompanied by advances in formulations and deployment technologies and the often-observed absence or disregard of evidence-based deployment rules and operating procedures, is of great concern. Epidemiological research on tear gas health effects is clearly deficient and has received little public support. Evidence from the limited epidemiological studies available and from case studies demonstrates that tear gas agents have the potential to cause serious harm and present specific threats to vulnerable populations, including children, women, and individuals affected by respiratory, cutaneous, and cardiovascular morbidities. While breakthroughs in mechanistic basic science have been made, discovering TRPV1 and TRPA1 as agent receptors, evidence that these targets are involved in multiple morbidities has not been taken into consideration for reassessment of tear gas use, and mechanism-based countermeasures development has not progressed.

Based on these deficiencies, we make the following recommendations:

(1) The toxicological effects of tear gas agents should be reassessed using state-of-the-art toxicological techniques utilizing toxicology in the 21st century (Tox21) collaboration approaches (https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21) and animal models of respiratory, cutaneous, and cardiovascular morbidities, taking into account age and sex differences.
(2) Epidemiological research networks should be established to develop standardized questionnaires; collect medical data, tissue, and fluid samples from exposed patients; curate biorepositories; perform environmental analysis; and conduct follow-up investigations. Mandatory cooperation of law enforcement with this network before, during, and after deployment needs to be specified in standard operating procedures.
(3) Epidemiological investigation of tear gas–exposed military populations should be extended to identify potential long-term effects.
(4) Efficient countermeasures need to be developed for treatment of individuals exposed to high levels of tear gas agents. Countermeasures should include new decontamination strategies based on the chemical properties of tear gas agents, their solvents, and pyrotechnic products and novel pharmacological inhibitors of the TRP ion channels TRPV1 and TRPA1, currently in clinical trials for pain indications.
(5) Efforts must be made to make tear gas munitions traceable to document use volume, deployment locations, and numbers.
(6) Efficient export and world trade controls must be enacted to prevent procurement and manufacture of RCAs by state actors and organizations that repeatedly deploy tear gas agents resulting in deaths, mass injuries, and widespread contamination and prohibit medical care and act against medical personnel treating the exposed.

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Conflicts of interest

Sven-Eric Jordt is serving on the Scientific Advisory Board of Hydra Biosciences Inc., a biopharmaceutical company developing TRP ion channel inhibitors for the treatment of pain and inflammation.

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O-Chlorobenzylidene Malononitrile (CS Riot Control Agent) Exposure in a U.S. Army Basic Combat Training Cohort

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Abstract **All U.S. Army soldiers participate in mask confidence training during initial military training and periodically throughout their careers. Training is conducted by dispersing the riot control agent, o-chlorobenzylidene malononitrile (CS), in a relatively air-tight structure where soldiers enter and conduct a series of exercises that culminate with mask removal. The study described here quantified CS concentrations experienced by 6,723 trainees and seven chamber operators during U.S. Army basic combat training at Fort Jackson, South Carolina, from August 1 to September 25, 2012. All 6,723 trainees were potentially exposed to CS concentrations exceeding the American Conference of Governmental Industrial Hygienists threshold limit value-ceiling (TLV-C) (0.39 mg/m^3), 6,589 of which were potentially exposed to concentrations exceeding the value deemed immediately dangerous to life and health (IDLH) (2.0 mg/m^3) by the National Institute for Occupational Safety and Health. All chamber operators were exposed to concentrations exceeding both the TLV-C and the IDLH.**

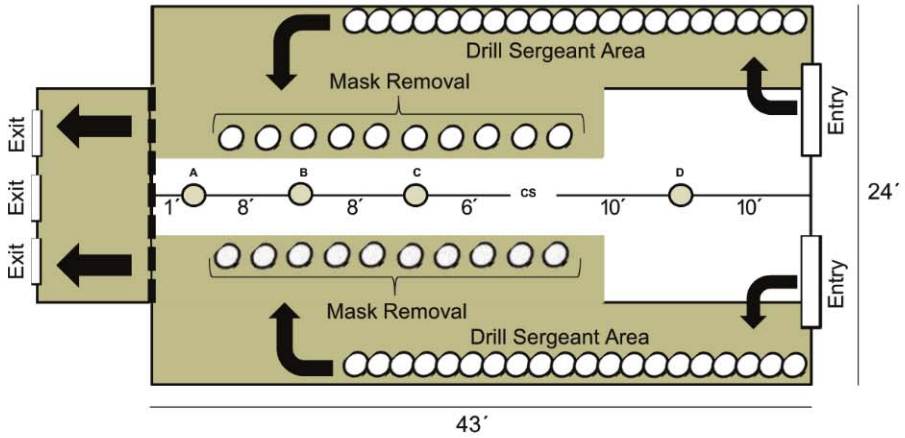
Introduction

O-chlorobenzylidene malononitrile (CS), commonly referred to as "tear gas," is an incapacitating agent used by military and law enforcement communities for training and riot control operations (Hout, Lapuma, Hook, & White, 2010). The incapacitating effects of CS are well documented (Archuleta & Stocum, 1993; Blain, 2003; Punte, Owens, & Gutentag, 1963; Salem et al., 2008; Sivathasan, 2010; Thomas, Smith, Rascona, Louthan, & Gumpert, 2002). The U.S. Army exploits these effects to provide realism to combat training events, to validate the serviceability of chemical protective equipment, and to demonstrate protection afforded by the chemical protective mask when challenged by airborne chemical agents (Department of the Army, 1996, 2012a). All new recruits entering the U.S. Army are exposed to CS during the first month of basic combat training (BCT) while participating in chemical, biological, radiological, and nuclear (CBRN) mask confidence training (MCT). Completion of the mask confidence chamber is a graduation requirement (Department of the Army, 2012b). In addition, all U.S. Army soldiers issued a protective mask must complete MCT on an annual basis (Department of the Army, 2011, 2012a). These two factors make CS a common exposure for many of the nearly 550,000 soldiers serving in the U.S. Army (Department of Defense, 2012).

MCT requires participants to enter an enclosed, CS-rich environment created by thermally dispersing CS capsules (Department of Defense identification code K765) in a relatively air-tight structure (Department of the Army, 2012a). Dispersal is controlled by a chamber operator wearing a qualitatively fit-tested M40 series military protective mask equipped with a C2A1 canister. The M40 is a full-face air purifying respirator (APR) that is specifically designed to protect the eyes, face, and respiratory tract from airborne chemical warfare agents when used in conjunction with the C2A1 canister (Department of the Army, 1994a; U.S. Army Chemical School, 2003). The C2A1 canister consists of a high-efficiency particulate air filter, followed by an activated carbon filter impregnated with copper, zinc, silver, molybdenum, and triethylenediamine, which is designed to remove chemical warfare agents and radioactive fallout particles from air entering the mask (Morrison, 2001). The chamber operator builds an initial CS concentration by thermally dispersing one 650-mg CS capsule on the surface of an empty heated coffee can for every 30 m^3 of chamber volume (Department of the Army, 1982, 1994b; Hout et al., 2010). Once the initial concentration is established, participants enter the chamber wearing the army combat uniform (ACU) (a 50/50 cotton/nylon digital camouflage patterned blouse and trouser that covers the body excluding the hands, wrists, neck and head, worn in conjunction with leather boots) and a qualitatively fit-tested M40 with C2A1 canister (U.S. Army Program Executive Office Soldier, 2012). Trainees conduct a series of physical exercises, break and reseal the air-tight seal between their mask and face, and finally line

FIGURE **1**

Fort Jackson Mask Confidence Chamber



Dark circles A–D represent sampling locations; light circles represent trainees; white represents the area open to sampling; and arrows depict the flow of trainees through the chamber.

up in groups of 10 to completely remove their masks before exiting the chamber. Chamber operators remain inside the chamber for the duration of the exercise, maintaining the CS concentration by dispersing an additional capsule for every 10 soldiers who pass through the chamber (Department of the Army, 1982, 2008, 2012a; Hout, White, Kluchinsky, & Lapuma, 2011). Participants experience an intense burning sensation on exposed skin and after removing the mask, almost immediate lacrimation, coughing, and sometimes vomiting. These events may occur earlier if the mask is defective or improperly fit. Absence of symptoms prior to mask removal develops confidence in the ability of the M40 mask to protect the user from airborne chemical agents (Department of the Army, 1982, 2008).

A 2010 study conducted at the Uniformed Services University of the Health Sciences (USU) demonstrated that low-temperature dispersal of CS capsules in an unoccupied mask confidence chamber and in a temperature-controlled tube furnace resulted in the formation of at least 17 thermal degradation products, some of which were hazardous to human health (Hout et al., 2010). A follow-on study conducted in an unoccupied mask confidence chamber showed CS dispersed in accordance with U.S. Army MCT guidelines resulted in CS concentrations exceeding the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value-ceiling (skin) (TLV-C), the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit-ceiling (REL-C), and the level that NIOSH deems immediately dangerous to life and health (IDLH) (Hout et al., 2011). These studies illustrate the potential for exposures to CS thermal degradation products and high levels of CS in a U.S. Army mask confidence chamber; however, they were not conducted during live MCT and thus did not represent a population exposure.

The current observational study quantified CS exposures in a cohort of U.S. Army BCT trainees (n = 6,723) and chamber operators (n = 7) from August 1, 2012, to September 25, 2012, during BCT at Fort Jackson, South Carolina, and compared them to published exposure guidelines. The study protocol was approved by the U.S. Army Training and Doctrine Command and the USU Office of Research, and was deemed nonhuman subjects research by the USU institutional review board.

Methods

The Fort Jackson mask confidence chamber is a 255-m^3 structure used solely for MCT. The chamber has two entrances and three exits at opposing ends of the structure covered by plastic strip curtains to prevent the escape of aerosolized CS (Figure 1). The floor is concrete, the walls are cinder block, and the ceiling is painted plywood. Chamber operators

Hot plate method of CS dispersion at the Fort Jackson mask confidence chamber.

establish an initial CS concentration by placing an empty 387-g coffee can on the small burner of a 1500-watt dual burner hot plate elevated on a 1.1-m tall table in the center of the chamber (see photo above). The hot plate is set to high (mean = 199°C) and the coffee can is preheated for approximately five minutes. Calculations showed that 8.5 capsules were required to establish the initial CS concentration; however, chamber operators consistently add 10 CS capsules and agitate and mix with a stirring rod until all visible CS is aerosolized (approximately five minutes).

Exposure groups (mean = 50 trainees) enter the chamber through both entrances, line up against the walls, and conduct a series of exercises that include breathing normally, breathing deeply, turning head from side-to-side, moving head up and down, rotating chin, running in place for 60 seconds, pulling mask away from the face, clearing the inside of the mask of airborne contaminants, and resealing the mask. Trainees then line up in groups of 10 at two of the exits, remove their protective masks, recite phrases chosen by the instructors, and exit the chamber (Figure 1). Once all trainees in an exposure group exit, a new exposure group immediately enters. One CS capsule is added for every 10 trainees who exit the chamber in the previous exposure group and the training continues as previously described. A maximum of 34 capsules are used for a military company consisting of four exposure groups.

Chamber Characterization

Initial sampling characterized CS dispersal within the chamber to determine the number and placement of sampling devices. Sampling was restricted to the low-traffic area that traversed the chamber from the entrances to the

TABLE **1**

CS[a] Concentrations and Exposure Durations for Trainees

Company #	Exposure Group 1			Exposure Group 2			Exposure Group 3			Exposure Group 4		
	n	Time (min.)	CS (mg/m^3)	*n*	Time (min.)	CS (mg/m^3)	*n*	Time (min.)	CS (mg/m^3)	*n*	Time (min.)	CS (mg/m^3)
1	55	10.1	**13.0**	53	8.5	**17.2**	56	10.0	**12.7**	54	10.0	**5.3**
2	47	10.0	**7.3**	47	8.7	**7.7**	46	8.0	**8.3**	45	8.0	**7.4**
3	55	9.0	**53.3***	55	8.0	**12.6**	52	7.0	**12.4**	56	8.0	**10.0**
4	55	7.5	**20.4**	56	6.5	**45.1***	54	8.0	**43.3***	55	10.0	**23.3***
5	53	9.0	**5.0**	55	7.5	**5.3**	48	7.0	**5.3**	54	8.5	**5.0**
6	50	9.0	**25.9***	49	9.0	**48.2***	50	10.0	**29.3***	49	10.0	**20.0***
7	45	5.5	**14.9**	48	7.0	**13.1**	50	10.0	**24.0***	44	10.0	**12.5**
8	51	9.0	**3.6**	53	7.0	**7.8**	55	10.5	**11.2**	52	10.5	**6.2**
9	51	6.0	**9.8**	51	7.0	**12.5**	52	6.0	**17.9**	50	5.0	**9.9**
10	51	7.0	**9.0**	51	7.5	**5.4**	57	8.5	**4.7**	55	10.0	**4.1**
11	52	9.0	**19.6**	52	10.0	**34.0***	51	10.5	**55.2***	51	10.0	**7.8**
12	43	10.0	**20.1***	45	10.0	**25.0***	41	9.0	**24.2***	44	10.0	**16.6**
13	53	8.5	**4.2**	51	7.5	**4.5**	56	8.0	**5.3**	56	10.0	**5.3**
14	60	7.5	**3.3**	55	8.0	**2.6**	52	7.0	**3.2**	63	10.5	**2.6**
15	57	7.5	**5.7**	59	6.5	**8.4**	60	10.5	**8.1**	58	10.0	**6.4**
16	67	10.5	**6.2**	68	9.0	**6.0**	67	8.5	**5.7**	No exposure group		
17	48	7.5	**5.2**	46	8.5	**4.4**	49	8.5	**4.0**	47	6.5	**3.5**
18	48	8.0	**3.9**	48	10.0	**4.0**	48	8.5	**3.6**	49	9.5	**4.0**
19	47	7.0	**6.0**	46	7.0	**2.9**	45	8.0	**3.3**	46	10.0	**3.0**
20	40	10.0	*1.7*	41	8.0	**4.4**	39	8.0	**5.1**	45	6.0	**2.8**
21	48	9.0	**18.5**	46	7.0	**10.1**	46	6.5	**7.6**	43	8.0	**7.0**
22	38	8.0	**5.0**	39	7.5	**4.5**	40	6.5	**2.6**	46	8.0	**4.1**
23	48	7.0	**8.8**	47	7.0	**8.0**	44	6.5	**7.3**	48	8.5	**6.6**
24	48	8.0	**3.6**	51	9.0	**11.4**	48	9.0	**7.7**	8	7.0	**3.2**
25	47	10.0	*1.9*	49	10.0	**5.7**	49	10.0	**5.7**	53	8.5	**8.6**
26	51	10.0	**2.7**	49	10.5	**13.1**	45	11.0	**13.9**	49	11.5	**7.5**
27	39	7.5	**6.7**	50	10.5	**6.4**	53	8.0	**6.6**	50	8.0	**7.2**
28	53	9.0	**19.3**	54	11.0	**12.3**	43	8.5	**10.4**	70	15.0	**16.9***
29	45	7.0	**4.1**	44	7.5	**3.8**	57	8.5	**4.9**	58	8.5	**5.6**
30	43	6.5	**2.6**	47	6.0	*1.8*	50	6.0	**4.7**	64	7.5	**5.5**
31	54	8.5	**6.9**	49	9.0	**5.5**	46	9.0	**4.0**	51	11.0	**5.6**
32	59	13.0	**3.6**	61	13.0	**5.6**	65	13.0	**7.7**	No exposure group		
33	46	9.0	**3.3**	47	9.5	**2.2**	49	9.5	**3.1**	50	11.5	**2.9**
34	46	10.0	**7.3**	55	9.5	**7.0**	57	9.0	**6.4**	29	7.0	**5.1**

Note. Values in italics exceeded American Conference of Governmental Industrial Hygienists' threshold limit value-ceiling (0.39 mg/m^3). Values in bold exceeded the National Institute for Occupational Safety and Health's immediately dangerous to life and health value (2.0 mg/m^3).

[a]CS = o-chlorobenzylidene malononitrile.

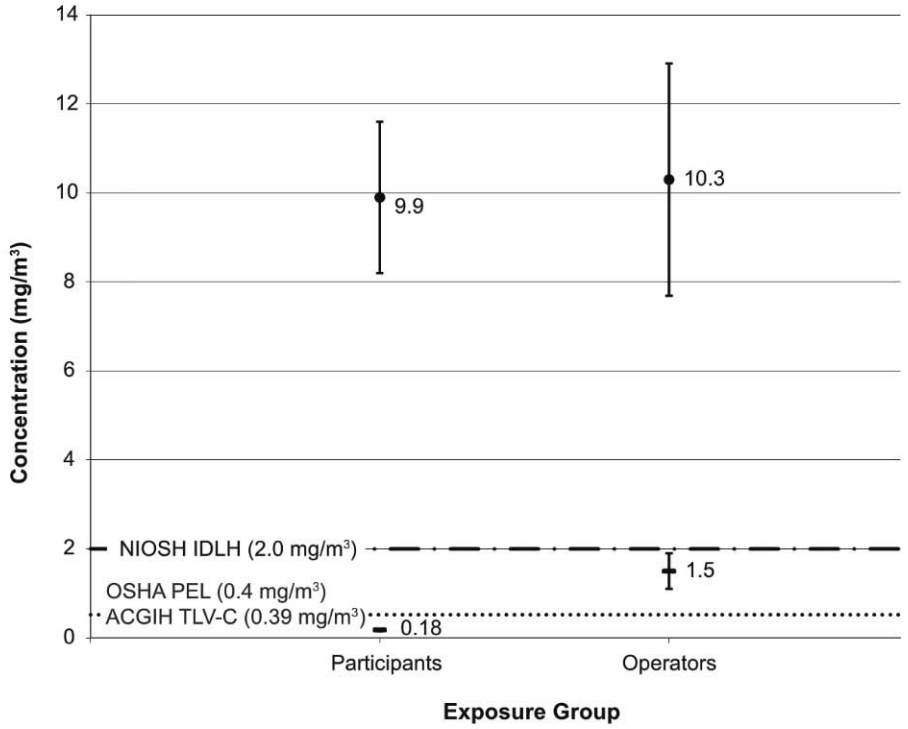
*Exceeded the Occupational Safety and Health Administration permissible exposure limit (0.4 mg/m^3) when averaged over the eight-hour work day.

exits. Sampling locations were selected to minimize training interference, provide representative respiratory exposure locations, and allow access to sampling media for rapid exchange. As shown in Figure 1, four initial sampling locations are based upon these criteria, three in the mask removal (A–C) area and one near the entrance (D).

The Occupational Safety and Health Administration (OSHA)–modified NIOSH physical and chemical analytical method 304 was used to sample for CS (OSHA, 2013a). This method uses an OSHA versatile sampler (OVS) tube in lieu of the NIOSH-required 37-mm polytetrafluorethylene with an in-line Tenax TA sorbent tube (National Institute for Occupational Safety and Health [NIOSH], 1979). The OVS sampler combines a glass filter with a two-sec-

FIGURE **2**

CS Concentration by Exposure Group



● represents mean exposure concentration; — represents mean eight-hour time-weighted averages; error bars represent 95% confidence interval.

CS = o-chlorobenzylidene malononitrile; NIOSH IDLH = National Institute for Occupational Safety and Health immediately dangerous to life and health; OSHA PEL = Occupational Safety and Health Administration permissible exposure limit; ACGIH TLV-C = American Conference of Governmental and Industrial Hygienists threshold limit value-ceiling.

tion sorbent bed (140/70 Tenax) in one tube to capture both the aerosol and vapor phases of CS. Sampling trains consisted of a 1.4-m section of Tygon tubing connected to OVS tube covers with OVS samplers inserted. Sampling trains were then connected to Aircheck XR5000 pumps calibrated to 1.5 L per minute using a BIOS DryCal.

OVS samplers were suspended 1.37 m above the floor on sampling stands at locations A–D to represent human breathing zone exposures. Pumps were sequentially started when an exposure group entered the chamber and sequentially paused after the exposure group exited. OVS tubes were removed, capped, sealed in individual 0.5-L plastic bags, and placed outside the chamber in an ice-filled cooler. A new OVS tube was then placed in each sampling train and sampling continued as previously described until all exposure groups completed training. Post-sampling flow rates were documented following each sampling event. OVS tubes were packed in ice and shipped within 24 hours via overnight mail to a certified laboratory for analysis. Sample size calculation results required 48 samples (12 from each location) to detect a 0.50 mg/m^3 difference between the sampling locations with 80% power at the 95% confidence level.

Exposure Assessment

Once the chamber was characterized, the most appropriate locations for CS sampling were determined for characterizing personnel exposures. Personal monitoring of trainees was not conducted due to potential training disruption. It is acceptable, however, to use area samples taken from a fixed location to represent exposures to multiple workers (OSHA, 2013a). Thus, area-based static sampling provided estimated trainee exposure concentrations. Sampling methodology was consistent with that used during chamber characterization. Thirty-four military companies comprised of a total of 6,723 trainees were involved in the exposure assessment. Thirty-two of the companies had four exposure groups, while two smaller companies had only three exposure groups. A total of 134 area samples were obtained to represent these exposure groups.

Operators remaining inside the chamber for the duration of the exercise wore personal monitors with a sampling train and flow rate consistent with area monitors. The OVS sampler was clipped to the chamber operator's lapel within six inches of the nose; the sampling pump was attached to the operator's belt. The pump was started immediately upon entry into the chamber and was not stopped until the training event was complete and the operator exited the chamber. A total of seven operators were monitored and 33 samples were obtained from these operators during their chamber exposures.

Results

Chamber Characterization

CS concentrations ranged from 0.4 to 53.3 mg/m^3 (mean = 10.4 mg/m^3) for 48 total samples, 12 from each location (Figure 1, A–D). The Shapiro-Wilk test indicated that the data were not normally distributed ($p < .01$) and required nonparametric analysis (Shapiro & Wilk, 1965). CS concentrations at sampling locations A–D were not statistically different when compared using the Kruskal-Wallis one way analysis of variance ($p = .198$) (Kruskal & Wallis, 1952). This is likely the result of mixing created by the constant movement of the trainees and chamber operators. These data suggest that CS is evenly dispersed across the four sampling points and allows one area sample at a fixed location (Figure 1, A–D) to represent chamber concentrations. Location C was selected as the location nearest the center of the mask removal area.

Exposure Assessment

Table 1 shows the 134 area samples used as individual exposure surrogate measures for the 6,723 trainees in our study. Observed CS concentrations were not normally distributed ($p < .01$) and ranged from 1.74 to 55.24 mg/m^3 (mean = 9.9 mg/m^3) with exposure durations and sampling times ranging from 5.0 to 15.0 minutes (mean = 8.7 min.). Eight-hour

time-weighted averages (TWA) ranged from 0.02 to 1.21 mg/m^3 (mean = 0.18 mg/m^3). All area samples (N = 134) exceeded the ACGIH TLV-C (skin) (0.39 mg/m^3), 98% (n = 131) exceeded the IDLH (2.0 mg/m^3), and 11% (n = 15) exceeded the OSHA permissible exposure limit (PEL) (0.4 mg/m^3). All trainees (n = 6,723) in the cohort were potentially exposed to CS concentrations exceeding the ACGIH TLV-C (skin), 6,589 to concentrations exceeding the IDLH, and 770 to concentrations exceeding the OSHA PEL (Figure 2).

The results of the personal monitoring samples collected from the seven different chamber operators (A–G) are displayed in Table 2. One data point was excluded from the analysis because of a pump failure. Personal monitoring samples were not normally distributed (p < .01) and ranged from 2.37 to 35.07 mg/m^3 (mean = 10.3 mg/m^3) with exposure durations ranging from 28.0 to 90.0 minutes (mean = 56.5 min.). All samples exceeded the ACGIH TLV-C (skin) and the IDLH. The eight-hour TWA for the chamber operators ranged from 0.3 to 5.0 mg/m^3 (mean = 1.5 mg/m^3); 32 of 33 samples exceeded the OSHA PEL (Figure 2).

TABLE **2**

CS[a] Concentrations, Exposure Durations, and TWA[b] for Chamber Operators

Company #	Chamber Operator				Company #	Chamber Operator			
	ID	Time (min.)	CS (mg/m^3)	TWA (8 hr.)		ID	Time (min.)	CS (mg/m^3)	TWA (8 hr.)
1	B	70.0	**11.1**	*1.6*	18*	D	57.0	**2.8**	*0.6*
2	C	48.0	**6.8**	*0.7*	19	D	46.0	**10.0**	*1.0*
3	A	54.0	**7.7**	*0.9*	20	E	51.0	**3.6**	*0.4*
4	A	54.0	**32.2**	**3.6**	21	G	Pump failure		
5	C	53.0	**6.5**	*0.7*	22*	D	49.0	**2.4**	*1.1*
6	A	69.0	**35.1**	**5.0**	23*	D	41.0	**10.1**	*1.1*
7	A	28.0	**15.0**	*0.9*	24	E	50.0	**9.9**	*1.0*
8	A	83.0	**9.1**	*1.6*	25*	F	64.0	**12.7**	**2.6**
9	A	83.0	**15.7**	**2.7**	26*	F	71.0	**6.1**	**2.6**
10	A	54.0	**10.3**	*1.2*	27	D	50.0	**10.5**	*1.1*
11	B	90.0	**21.4**	**4.0**	28	F	62.0	**15.1**	*2.0*
12	A	87.0	**19.5**	**3.5**	29	D	50.0	**3.3**	*0.3*
13*	E	52.0	**7.3**	*1.2*	30	D	41.0	**6.0**	*0.5*
14*	E	56.0	**3.1**	*1.2*	31	G	46.0	**5.4**	*0.5*
15	E	60.0	**9.7**	*1.2*	32	F	47.0	**6.1**	*0.6*
16	D	46.0	**11.5**	*1.1*	33	G	55.0	**5.0**	*0.6*
17*	D	50.0	**2.9**	*0.6*	34	D	48.0	**6.7**	*0.7*

Note. Values in italics exceeded American Conference of Governmental Industrial Hygienists' threshold limit value-ceiling (0.39 mg/m^3). Values in bold exceeded the National Institute for Occupational Safety and Health's immediately dangerous to life and health value (2.0 mg/m^3).

[a]CS = o-chlorobenzylidene malononitrile.

[b]TWAs = time-weighted averages.

*Companies 13 and 14, 17 and 18, 22 and 23, and 25 and 26 occurred in the same eight-hour workday, respectively.

Discussion

U.S. Army doctrine dictates the exposure standards set forth by OSHA will be adhered to unless the exposure standards set by ACGIH are more protective; the ACGIH TLV-C, the OSHA PEL, and the NIOSH IDLH are applicable to CS exposures (Department of the Army, 2005). The TLV-C (0.39 mg/m^3) is a value that should not be exceeded during any part of the exposure scenario and was established to minimize CS-induced damage to the respiratory epithelium and protect against symptoms including burning of exposed skin and potential skin sensitization (American Conference of Governmental Industrial Hygienists, 1991, 2010). The OSHA PEL (0.4 mg/m^3) is the average concentration exposure during an eight-hour work period that should not be exceeded during a 40-hour work week and was developed to reduce the risk associated with skin, eye, and respiratory effects (Air Contaminants, 2006; OSHA, 1995, 2013b). The NIOSH IDLH (2.0 mg/m^3) was established to prevent delayed or permanent health effects (including death) associated with exposure and to protect against eye, respiratory, and other effects that could prevent escape from the exposure scenario (NIOSH, 1994).

The primary routes of trainee exposure are inhalation (respiratory tract) and absorption (skin and eyes) (OSHA, 1976). Exposures to the respiratory tract and eyes may have occurred if a mask was defective or improperly sealed; when required to break the seal of the mask; or when required to remove the mask prior to exiting the chamber. Army safety guidelines state, "Unprotected personnel will not be exposed to riot control agents longer than 15 seconds;" however, observed time out of mask for 34 randomly selected participants from different companies ranged from 29 to 122 seconds (mean = 48.9 s) (Department of the Army, 2012c). Skin exposures occur continuously with hands, wrists, necks, and backs of the head fully exposed to airborne CS. ACGIH advises that when a chemical bears a skin notation, measures should be taken to prevent dermal contact because air sampling does not account for exposure contributions via the cutaneous route (ACGIH, 2010). Furthermore, CS penetrates and remains in uniform fabric, presenting potential longer-term exposures. These factors suggest that trainees are potentially exposed to CS at levels greater than those indicated by the air monitoring results presented here.

It is common practice to monitor workers closest to the point of generation (chamber operators) with the assumption of a worst-case exposure scenario (OSHA, 1985). Since the data presented here show chamber operators are overexposed, the potential for similar trainee overexposure is possible and consistent with the area sampling data presented in Table 1. It is important to note, however, that full-period sampling was used to monitor the chamber operators. This sampling method-

ology provides the mean concentration each chamber operator was exposed to during a particular chamber exercise. Changes in concentrations created by addition of CS, doors opening, trainees exiting, and general mixing within the chamber are not individually captured, but averaged together. Consequently, chamber operators may have been exposed to higher CS concentrations than are reported here. Conversely, trainees had much shorter sampling durations that may have captured many of the aforementioned concentration changes. The difference in sampling durations resulted in a disparity in the observed concentration ranges for trainees versus chamber operators; however, the Mann-Whitney U test failed to reveal a statistical difference between the mean trainee CS concentration (mean = 9.9 mg/m^3) and the mean chamber operator CS concentration (mean = 10.3 mg/m^3) at the 95% confidence level (p = .172).

Chamber operators are not required to break the seal or remove their mask; however, the question of respirator efficacy in this environment remains. The M40 is designed to protect the respiratory system from military chemical warfare agents. The Department of the Army and Department of Defense are the approval authorities for respirators to be used for protection against these agents; however, riot control agents are specifically exempt from this definition, leaving NIOSH as the approving authority (Department of the Army, 2013). A quantitatively fit NIOSH-approved full-face APR has an assigned protection factor (APF) of 50 and is capable to protect against airborne concentrations up to the IDLH; however, the M40 is not NIOSH approved and thus does not have an APF. Without an APF, it is difficult to determine whether the M40 provides adequate respiratory protection for concentrations approaching the IDLH level. When IDLH is exceeded, a full-face pressure demand self-contained breathing apparatus or a combination full-face pressure demand supplied air respirator with auxiliary self-contained air supply is required (Bolinger, 2004; Department of the Army and Defense Logistics Agency, 1982; OSHA, 1976; Respiratory Protection, 2006). Routine entry into this type of environment requires approval from either the installation medical authority or the safety and occupational health manager (Department of the Army, 2013). Since chamber operators are exposed to levels exceeding the PEL and IDLH on a routine basis, they should be issued a NIOSH-approved quantitatively fit respirator and be enrolled in a respiratory protection program (Department of the Army, 2007, 2013; Department of the Army and Defense Logistics Agency, 1982; OSHA, 1995; Respiratory Protection, 2006). All chamber operators in our study wore only a qualitatively fit-tested M40 series protective mask and were not enrolled in a respiratory protection program.

Chamber operators were also subjected to the effects associated with dermal exposure to CS. Three of the seven chamber operators wore the mission-oriented protective posture (MOPP) level four ensemble without the chemical protective over boots. MOPP level four features a chemical protective over garment that covers the upper and lower body, chemical protective gloves and over boots, and the M40 series mask with attached hood (Rimpel, Boehm, O'Hern, Dashiell, & Tracy, 2008; U.S. Army Chemical School, 2003). The remaining four chamber operators (and three investigators) wore only the ACU and an M40 protective mask without a hood. Three of four chamber operators and two of three investigators who wore only the ACU and M40 protective mask without the hood developed erythema that persisted for up to 48 hours on the exposed skin on the back of the neck and head. These reactions are consistent with those from prolonged CS skin exposures (Archuleta & Stocum, 1993; Blain, 2003; Shmunes & Taylor, 1973).

Conclusion and Recommendations

Our study is the first to quantify CS exposures in U.S. Army BCT trainees and chamber operators. Both cohorts were potentially exposed to CS levels requiring a greater level of respiratory and skin protection than afforded at the time of our study. All members of the BCT cohort were potentially exposed to CS concentrations exceeding the TLV-C (skin), 98% of which exceeded IDLH. This is consistent with previous unoccupied chamber studies that suggested the U.S. Army MCT procedures produce CS concentrations exceeding guidelines established by ACGIH, NIOSH, and OSHA (Hout et al., 2011).

A work practice control of decreasing the concentration of CS used in the MCT may reduce the potential for overexposure to CS. MCT's primary goal of demonstrating the capability of the protective mask can be accomplished using CS concentrations bounded by the odor threshold (0.004 mg/m^3) and the TLV-C (0.39 mg/m^3) (Department of the Army, 1996; Hout et al., 2010). If concentrations remain within this range, the need for skin and respiratory protection is greatly decreased. Chamber CS concentrations should be evaluated by industrial hygiene personnel at least annually to verify exposure levels (Department of the Army, 2007). Pairing this with administrative controls such as rotating chamber operators and limiting the time trainees are inside the chamber without respiratory protection to 15 seconds should significantly reduce overexposure potential.

If CS concentrations are not reduced, personal protective equipment must be relied upon to reduce exposures. The use of the chemical protective garment ensemble by both the trainees and operators should reduce potential skin exposures. Respiratory exposures may be minimized through proper mask maintenance, quantitative fit testing, and equipping chamber operators with NIOSH-certified masks designed to protect them against levels of CS exceeding IDLH. Chamber operators should also be enrolled in a respiratory protection program.

The results of our study prompted the March 2013 publication of All Army Activities (ALARACT) message 051/2013, which incorporated several of the controls recommended here into future army MCT events. Specifically, it reduced the number of CS capsules required to charge the chamber, reduced the number of capsules used to maintain the CS concentration, and specified a maximum time out of mask of 15 seconds. It also mandated semiannual industrial hygiene assessments of all army mask confidence chambers and periodic wet cleaning of said chambers (U.S. Army Safety Office, 2013).

Ongoing research is being conducted to investigate health effects associated with the CS exposures documented here. Future research is needed to quantify CS exposure levels after implementation of ALARACT 051/2013 to determine if the controls were effective in lowering CS concentrations and to study the effect of these controls on health outcomes.

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